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Thesis
1964 (F)
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FINE STRUCTURE OF SKIN, LATERAL-LINE
ORGANS AND MELANOPHORES OF FROG TADPOLE

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read,
and recommend to the Faculty of Graduate Studies for
acceptance, a thesis entitled Fine Structure of Skin,
Lateral-line Organs and Melanophores of Frog Tadpole
submitted by Sohan Singh Jande in partial fulfilment of
the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The skin of frog tadpole (Rana pipiens) consists of an epidermis underlain by a basement lamella to which it is affixed by a dermo-epidermal junction. The epidermis consists of two layers of cells. Whereas the cells of the outer layer are engaged in the secretion of mucus (mucoproteins), the cells of the basal layer are specialized for the union of epidermis with the basement lamella.

The adepidermal space lying between the plasma membranes of the basal cells and the dermal membrane is occupied by a cementing substance which is quite labile to various fixatives. The nature of this substance, on the basis of its appearance using different fixatives and of the effects of hyaluronidase, seems to be mucoprotein. The basement lamella, consisting of an array of collagen fibrils embedded in a ground substance, increases in thickness by the addition of new layers from the epidermal side.

Chlorinated tapwater removes epidermis from tadpoles by affecting the sulfhydryl enzymes and other cell constituents liable to oxidation. This disrupts the barriers which normally prevent the inflow of water into the animal with the result that intercellular spaces increase and push apart the outer layer of cells which peel off in sheets.

Four types of cells can be distinguished in the lateral-line organ on the basis of their function, form and position. The receptor cells which are concerned with the perception of stimuli differ markedly in their fine structure from others which are mainly supportive in function. The

latter, however, resemble each other in their fine structure and are engaged in the secretion of some material into intercellular spaces in the organ. The cells which have their bases affixed to the basement lamella show bobbins.

Microtubules, so far not known in receptor cells, have been observed in the receptor cells and it is hypothesized that these play an important role in the initiation of nerve impulses in the receptor cell associated nerve endings. It appears that the impulse initiation is caused by the release of a chemical transmitter substance.

Afferent and efferent nerve endings have been distinguished on the basis of their fine structure.

The epidermal melanophores lie free in the epidermal intercellular spaces and contain lysosomes, dense bodies and keratin filaments in addition to melanin granules. The lysosomes show within them large numbers of melanin granules.

The dermal melanophores which are embedded in the dermal connective tissue are entirely melanin effector cells.

ACKNOWLEDGMENTS

I am greatly indebted to Dr. J. R. Nursall for suggesting to me this field of research, for his guidance and help in the interpretation of the findings and for improving the quality of the manuscript. I am thankful to Dr. D. M. Ross for his encouragement and kindness during my entire stay in this department. I am grateful to Dr. T. A. Shnitka for his unstinted help in the evaluation and interpretation of the electronmicrographs and for correcting the manuscript. I am thankful to Dr. G. O. Mackie for introducing me to electron microscopy. The technical help by Mr. G. Menges during all phases of study is gratefully acknowledged. I take this opportunity also to thank all the staff members of the department, both academic and technical as well as all the graduate students for their assistance and for making my stay in this department memorable.

Financial assistance was obtained from the National Research Council of Canada through research grants to Dr. J. R. Nursall and Dr. D. M. Ross.

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I. GENERAL INTRODUCTION

Biologists now have at their disposal an instrument, the electron microscope, which provides a resolution about two hundred times better than the best light microscope. This instrument, together with other modern techniques, has contributed a great deal in the understanding of various biological phenomena. The concept of cell structure and interrelationship of cells as understood today is not that which it was a decade ago. The use of the electron microscope in studying biological ultra-structure is thus imperative.

The present study was started in 1957 when a culture of frog tadpoles was accidentally introduced into tapwater and very drastic effects were noted on the skin of these creatures. To understand these changes it became necessary to study the structure of normal skin, about which little was known at the time. After the study of normal skin and the effects of chlorinated tapwater on it, the study was further continued to the examination of lateral-line organs and melanophores which are embedded in the skin. For convenience of description and analysis of the findings, the thesis has been sub-divided into three parts. The first part includes the structure of normal skin of tadpole and the effects of chlorinated tapwater. It also includes a brief study of the effects of hyaluronidase which throws some light on the nature of the cementing substance present between the cells. The second part provides a

description of the functional morphology of lateral-line organs. This study has provided a wealth of new findings which were previously unknown in various receptor organs. The third part comprises a study of structure of pigment cells, the melanophores, epidermal as well as dermal. Except for a very brief paper by Falk and Rhodin (1957) on Fundulus melanophores, there was no information available about the fine structure of the pigment cells, which play an important role in the survival of these organisms. The structure of these cells has been found to differ markedly from that described in previous studies.

II. MATERIAL AND METHODS

Electron microscopy:

Tadpoles of Rana pipiens (Schreber) were used as the material for this study. Adult frogs obtained from commercial suppliers were induced to spawn by pituitary gland injections after Rugh (1948). The tadpoles were reared at room temperature.

Throughout this study it was the tail of tadpole which was used because of the ease with which it can be cut into small pieces for preparation for electron microscopy.

Fixation:

Three different fixatives were used in the preparation of the material. These are osmium tetroxide, potassium permanganate and glutaraldehyde.

Osmium tetroxide was used as a one percent solution buffered by veronal acetate buffer after Palade (1952).

Potassium permanganate, which was first used as a fixative by Luft (1956), was used as an unbuffered three percent solution in distilled water.

Glutaraldehyde was first used as a fixative for electron microscopy by Sabatini, Bensch and Barnett (1962). In the present study it was employed as a three percent solution in Sorenson's buffer (pH7.4). The material was post-fixed in two percent osmium tetroxide solution in the same buffer. The material, after fixation with

THEORY

1. INTRODUCTION

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2. CONCLUSION

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The sixteenth part of the paper is devoted to a review of the literature.

glutaraldehyde, was washed for two hours in the buffer to which sucrose, in concentration of 10 percent, had been added. This washing is necessary before post-fixing in osmium tetroxide because glutaraldehyde reduces osmium tetroxide. The reduced osmium tetroxide is not effective as a fixative, and further the reaction product forms a coating on the outside of the tissue making the penetration of osmium tetroxide into tissue even slower than usual. Two concentrations of glutaraldehyde, three percent and six percent, in Sorenson's phosphate buffer, were tried in combination with osmium tetroxide for varied times. Best results were obtained with three percent glutaraldehyde used for two hours and two percent osmium tetroxide for one and a half hours.

Fixation was done at about 0° C. The vials containing the fixatives and material were kept in crushed ice made from distilled water.

Dehydration:

Ethanol was used for dehydration throughout this study. Acetone was tried but it did not yield any superior results and its use was discontinued. The material was dehydrated as per following schedule:

30% ethanol		10 minutes	
50"	"	"	"
70"	"	"	"
90"	"	"	"
100"	"	15	"
100"	"	"	"

The entire procedure of dehydration was done in the cold as was fixation. The material in the final 100% alcohol was allowed to come to room temperature before proceeding further.

Embedding:

Three different embedding media were tried. These were methacrylate, Araldite 502 and Epon 812. Methacrylate was tried in the beginning but its use was discontinued when vastly superior results were obtained with Araldite 502. For comparison Epon 812 was also tried, but it did not produce as desirable a result and so Araldite 502 was preferentially used.

Araldite was used as suggested by Luft (1961) with minor variations. A 50:50 mixture of dodecenyl-succinic anhydride (DDSA) and Araldite 502 was used and benzyl-dimethylamine was used as accelerator in concentration of one percent in the final plastic mixture. The embedding procedure that was followed is described below:

1. After dehydration, material was kept in two changes of propylene oxide for a total of 10 minutes.
2. Then it was transferred to a 50:50 mixture of fresh propylene oxide and plastic mixture (DDSA and Araldite 502 without any accelerator).
3. After one hour about the same volume of plastic mixture was again added and the material was left overnight in an uncovered vial.

4. The material was then transferred to the plastic mixture which contains 50:50 DDSA and Araldite to which accelerator had been added, in which it was left for about 12 hours at room temperature, before putting it in a 60° C oven for polymerization. It was left in an oven for at least 48 hours.

To ensure proper orientation of the material a few drops of plastic mixture (with accelerator) were polymerized at the bottom of the gelatine capsule (No. 00) prior to the transferrance of the material into these capsules.

Sections varying between 500 to 1000 Å were cut with Porter-Blum microtome (MT-I) using a glass or a diamond knife (Du Pont). The sections were collected on one percent solution of acetone in distilled water and were expanded by xylene vapours before being picked up on copper grids (150 and 200 mesh). As Araldite sections can withstand electron beams of medium intensity, uncoated grids were used.

The electron microscope used for the study was Phillips EM 100 B with an objective aperature of 25 . Micrographs were taken on 35 mm Kodak film (P426) at initial magnification of 1650 to 10,000. These were further enlarged photographically about six times.

Various heavy metal stains were used. Best results were obtained with a saturated solution of uranyl acetate, in water and in 50% ethanol, and lead hydroxide (Millonig, 1961). Staining with phosphotungstic acid during dehydration

was tried but results were very poor. KMnO_4 after Lawn (1960) was also tried but was not very useful in the present study.

Chlorine effects:

For the study of effects of chlorine on the skin, free-swimming tadpoles were used. At first tadpoles of Rana sylvatica Le Conte were used reared from eggs collected locally. Later, tadpoles of Rana pipiens Schreber were used. These were also reared from eggs as described earlier, obtained by induced breeding by pituitary gland injections.

The smallest tadpoles that were used were about 12 mm. in length; similar results could be obtained until the animals reached about 30 mm. In most experiments the animals were in the 15 to 25 mm. range of lengths. The condition of the epidermis on the tail was considered the primary indicator of necrotic reaction, chiefly because of ease in sectioning the tail and preparation of the serial mounts.

The active disinfectant in Edmonton city tapwater is chloramine. The concentration of residual chlorine at the tap is about one part per million although this varies according to needs at different times of the year. For experimental purposes residual chlorine was added to dechlorinated water by the addition of a solution of sodium hypochlorite (NaOCl). A liquid commercial bleach (Ajax) guaranteeing seven percent available chlorine provides replicable results. The chief experimental procedure was as follows:

Tadpoles were treated in lots of 10 in 250 ml. of test solution in glass finger bowls. Available chlorine was measured by means of a Hellige chlorine comparator at the beginning and end of each run. The double measurement was necessary because of the reactivity of chlorine; the concentration decreased drastically and rapidly. The reactivity of the chlorine made it impracticable to keep a stock of reagents. Test solutions were made to measure for each run. Temperature and pH were recorded.

Treated and control specimens were examined alive by means of a stereomicroscope and samples of both were fixed variously for histological examination. When necessary, particularly for electron microscopical examination, individual tadpoles were treated for specific lengths of time in specific concentrations of chlorine.

Hyaluronidase effects:

For the studies of the effects of hyaluronidase on tadpole skin, the free-swimming tadpoles, about 15 mm. in length were put directly in stabilized hyaluronidase (available as Wydase from John Wyeth & Bros. (Canada) Ltd.), each milliliter of solution containing 150 USP (T.R.) units. After one hour treatment, when the animal was still alive, the integument of the tadpole was fixed and prepared, as described before, for electron microscopy.

IIIa. STRUCTURE OF TADPOLE SKIN

INTRODUCTION:

Eberth (1866) was probably the first investigator to study the skin of the frog tadpole (Rana pipiens) microscopically. Other workers who later examined similar material (tadpole skins of various species of frogs) were Leydig (1879), Pfitzner (1882), Canini (1883), Mitrophanow (1884), Kolliker (1885), Macallum (1885), Frenkel (1886), Cohn (1895), Maurer (1895), Schuberg (1907, 1908), Studnika (1909), Lowenthal (1911), Saguchi (1913), Biedermann (1926), Speidel (1926), Weed (1934), Cameron (1936), Rosin (1946), and Mizuhira (1951). A summarized account of their findings is given below. This in fact is a brief description of the structure of tadpole (R. pipiens) skin as discerned by the light microscopists.

The epidermis of the tadpole, in the tail region, rests on a corium of dense connective tissue and consists of two strata of cells. The cells of the outer layer, those exposed to the outside, are not cornified as in adult frog skin but bear a cuticular border which extends across their outer surfaces and bridges over the intercellular spaces so that an unbroken surface is exposed to the outside. These cells were described to maintain a distinct identity from the lower cells, which have their bases attached to the corium below, although protoplasmic bridges connect the cells of these two layers in the same manner as all epidermal cells are linked.

The structures which have received the greatest attention, in the cells of the basal layer, were the coarse intracellular fibers or figures of Eberth. Weed (1934) described these to be made up of many fine fibrils having a tendency to adhere together to form coarse strands. He avoided such suggestions as that these represent secretory material (Leydig, 1879), intracellular nerve endings (Pfitzer, 1882; Canini, 1883 and Frenkel, 1886), sheaths of nerve endings (Macallum, 1885) and concluded, along with Cohn (1895), Schuberg (1907 and 1908), and Studnicka (1909), that these serve as supportive structures within the epidermal cells. He further maintained that these figures of Eberth are connective tissue branches from the corium and serve to bind the epidermis to the corium.

The epidermis is underlain by a basement membrane which consists of a hyaline ground substance with collagen fibers embedded in it. Rosin (1946) showed the orthogonality in the arrangement of these fibers.

With the advent of the electron microscope and development of the procedures for the preparation of biological material for examination with this instrument many workers undertook studies of the structure of skin, especially amphibian larval and mammalian skin.

Most workers have restricted their observations to small sub-units of the skin, except Porter (1954), who published a brief paper on the structure of animal epidermis based on the examination of various vertebrate animals but most especially on the skin of larval Amblystoma.

Weiss and Ferris (1954a, 1954b and 1956) used the tadpole of Rana clamitans and were concerned only with the complex array of collagen fibers in the basement lamella and with the site of dermo-epidermal junction.

Kemp (1959 and 1961) who used tadpoles of R. pipiens, studied the structure of basement lamella and followed the changes involved during the replacement of the larval basement lamella by adult type basement membrane.

Chapman and Dawson (1961) described briefly the structure of epidermis of R. clamitans with special reference to figures of Eberth. Singer and Salpeter (1961) also studied the figures of Eberth in three different species of Rana.

A more detailed work, however, has been done on the mammalian skin. Laden, Linden and Erickson (1953), Selby (1955) and Clark and Hibbs (1958) studied the structure of epidermal cells and dermo-epidermal junction. Odland (1958) studied the fine structure of interrelationship of cells in human epidermis. Brody (1959 and 1960) studied the keratinization of mammalian skin.

As mentioned earlier, the electron microscopic studies of skin especially those on the amphibian tadpoles were all restricted to its small aspects and were mainly concerned with the correction of various incongruities of the light microscope. An endeavour, therefore, will be made here to describe the structure of the skin of tadpole (R. pipiens) as a whole and also to discuss various intriguing

problems concerned with the skin in general such as the mechanism by which the dermo-epidermal junction is maintained, the production of collagen material and initiation as well as orientation of collagen fibers and also those concerned with tadpole skin such as the nature, mechanism and function of secretion of the secretory material produced by the cells of the outer layer.

OBSERVATIONS:

The larval skin (Figs. 1 and 2) consists of an epidermis comprising two layers of cells, underlain by a basement lamella (BL) consisting of an array of collagen fibrils embedded in a ground substance. The cells of the two layers of epidermis show a considerable structural differentiation. The structures of these two types of cells, the cells of outer layer and cells of basal layer, are described below.

Cells of outer layer:

The cells of the outer layer (OC), when seen in vertical section, are roughly triangular in form with their bases directed towards the outside (OS) but when observed in surface view are polygonal in shape (Fig. 3). Their nuclei (N) are flattened in the plane of the skin and appear oblong in a vertical section. The nuclear envelope shows invaginations. The cortex of these cells, especially towards the outside, is filled with a filamentous material. The 'filaments' (using the terminology advocated by Mercer et al, 1963) are about 60 Å in diameter. Because these do not follow a straight

course and pass out of the plane of the section their longitudinal dimensions, therefore, have not been ascertained. These filaments appear to be similar to those described in mammalian epidermis and referred to as keratin (Selby, 1955; Odland, 1958) .

In the cortex, showing a matrix of filamentous material towards the outside, are embedded large vesicles (MV) of varying sizes, which form almost a complete layer beneath the outer plasma membrane. Each vesicle is surrounded by a membrane around which the filaments form a sort of mesh-work. This is especially clear in Figs. 4 and 5. The latter is a section cut horizontal to the surface of skin. Some vesicles may be seen to be incompletely enveloped by a membrane. Such a condition seems to be an artifact. Sometimes vesicles containing similar contents are also found at a considerable distance in the interior of the cell. Fig. 6 shows one such vesicle which appears to be enlarging by a coalescence of smaller vesicles. These in turn seem to originate in the nearby Golgi region (G) .

These vesicles can even be seen with the light microscope especially when these are viewed in fresh material from the surface view using phase contrast (Fig. 3) . The contents of the vesicles present a vacuolated appearance in osmium tetroxide fixed material (Figs. 4-8) . But in the material fixed in glutaraldehyde it presents a homogeneous and quite electron opaque appearance (Figs. 1 and 9) . These vesicles are stainable with mucicarmine

(Carleton and Drury, 1957), which is generally used for the identification of mucus. These are also PAS positive, stain very faintly with Alcian blue, show a faintly positive Hale's dialysed iron reaction for acid mucopolysaccharides (Pearse, 1961) and do not show metachromasia with toluidine blue. The contents of the vesicles thus appear to be mucoprotein in nature.

As seen in Figs. 1, 2 and 4 the tops of most of these vesicles are in close apposition to the outer plasma membrane. Fig. 8 shows a vesicle discharging its contents to the outside. The membrane lining the vesicle can be seen to have become continuous with the outer plasma membrane. The contents of the vesicles form a layer on the surface of the outer cells where it presents a furry appearance.

The outer plasma membrane appears to be thicker and more electron opaque than that around the remainder of the cell. This is especially clear in Fig. 9, where both can be compared. The outer membrane is thrown into small folds about 0.1μ in thickness. These resemble microvilli in vertical section (Figs. 1, 2 and 6), but show their true nature in a section grazing the surface of the epidermal cells (Fig. 10). They are in fact microrugae, especially around the mucus vesicles.

These cells show quite a few mitochondria (M), which in 12 mm. larva are arranged in a layer towards the outer surface (Fig. 7). There is no such arrangement in the older larvae (Figs. 1 and 2). Each cell also shows a Golgi complex (G) which is a system of cisternae and

vesicles lined with smooth surfaced membranes (Fig. 11). The cisternae and vesicles contain slightly electron opaque contents in osmium tetroxide-fixed material but in glutaraldehyde-fixed material, the contents of vesicles as well as of the cisternae are intensely electron opaque (Fig. 12). The vesicles vary in size; some of them may be seen to coalesce with others to form larger vesicles (Fig. 11). There is also seen endoplasmic reticulum (ER) with its associated ribosomes. Some free ribosomes are visible as polysomes and a few lysosomes (LY) can be made out.

The outer cells are joined together to form a layer. At the site of union of two adjacent cells is present a protrusion about 0.2μ in thickness. Both cells contribute almost equally to its formation (Figs. 1 and 9). Deep to the apex of the protrusion the plasma membrane of contiguous cells are closely applied to each other for some distance leaving between them a uniform space about ^o125 Å in width. This resembles a terminal bar. Such a union is present all around the outer cells and seal off the intercellular spaces. Below this the union of two cells is reinforced by a series of desmosomes. A vertical section (Fig. 9) shows only a few of them but a horizontal section (Fig. 13) passing through this site of union shows that these are present all along the plasma membrane. The desmosomes are similar to those described by Selby (1955) and Odland (1958) in mammalian epidermis.

Cells of basal layer:

The cells of the basal layer appear roughly polygonal in a horizontal section (Fig. 14) but triangular with their bases resting on the corium below, in a vertical section (Figs. 1 and 2). Their apices interdigitate with those of the cells of the outer layer. The nuclei of these cells are also flattened in the plane of the skin and appear oblong in vertical section. In horizontal section the nuclei appear lobulated (Fig. 14).

The cortex of these cells is filled with filaments similar to those described in the outer cells. In the basal region the filaments are grouped together to form bundles (FE). Their one end is anchored to the basal plasma membrane and the other penetrates deep into the interior of the cell branching into smaller bundles. The branches intertwine with others in the manner of a skein. These are the 'figures of Eberth' and have been described in detail by Chapman and Dawson (1961) in the tadpole of Rana pipiens and by Singer and Salpeter (1961) in that of R. clamitans. Though these bundles have generally only one of their ends anchored to the basal plasma membrane as in Figs. 1, 2 and 15 but sometimes both ends of such a bundle may be seen attached to the basal plasma membrane at adjacent points (Fig. 16) forming a loop, or one end at basal plasma membrane and the other at a desmosome (Fig. 17). The plasma membrane at these places shows local differentiations in the form of thickenings (Fig. 15). Next to this thickened membrane (D_1), towards the interior of the cell is a zone of lighter density

which is further overlain by a denser region (D_2) which seems to be formed by close approximation of filaments of the bundle. This complex consisting of two dense regions separated by a lighter zone was designated as 'dense thickenings' by Porter (1954), as 'bobbins' by Weiss and Ferris (1954a), as 'half desmosomes' by Selby (1955) and Chapman and Dawson (1961) and 'half prickles' by Charles and Smiddy (1957). The basal plasma membrane between bobbins shows invaginations of varying sizes. This is the appearance of bobbins in osmium tetroxide fixed material. If, however, the material is fixed in unbuffered $KMnO_4$, the filaments converging on to the bobbins appear as amorphous material (Fig. 18), there is no trace of bobbins at all and the plasma membrane shows no thickening.

Around the nucleus are seen a few mitochondria, and a meager amount of endoplasmic reticulum with its associated ribosomes. There are also seen groups of ribosomes as polysomes (Figs. 1, 2 and 19). Round vesicles of about 600 Å in diameter can also be seen dispersed in the cell. These are very pronounced in potassium permanganate fixed material (Fig. 18).

Cells of the outer and the basal layer show a few intensely electron opaque melanin granules.

The basal cells throw out from all except the deep side protoplasmic protrusions which meet similar ones from other cells, basal or outer, depending on the situation, to form intercellular bridges (Figs. 1, 2 and 14). In about

the first of these is the fact that the system is not a simple one, and that the results of the experiments are not in agreement with the theoretical predictions.

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the middle of each such intercellular bridge a desmosome (DS) is always present. The bridges span quite large intercellular spaces (IS). Sometimes a few microvilli-like structures are seen projecting into the intercellular spaces.

Basement lamella:

Below the epidermis are present collagenous fibrils (in the terminology of Bear, 1952) arranged in layers (Figs. 1, 2 and 15). The fibrils of each layer run essentially in one direction and those of the successive layers run approximately at right angles to one another (Fig. 20). The number of the layers goes on increasing with the age of larva (Weiss and Ferris, 1954a). The maximum number of layers observed in the present material is about 23. In almost every preparation it has been observed that the fibrils in the uppermost layers, i.e. towards the epidermis, are uniformly smaller in diameter than those in the other layers, though occasionally such finer fibrils may also be seen amongst the comparatively larger ones (Fig. 15). The diameter of the largest fiber is about 350 \AA and that of the smallest is about 60 \AA , with others between these two extremes. All the fibrils show a periodic banding of about 750 \AA .

Occasionally this regular arrangement of collagen fibrils, with their long axes in the plane of the skin, is disturbed by bundles of fibrils running in vertical direction (Fig. 21).

Amongst the layers of collagen fibrils are sometimes seen round or oblong membrane-lined structures (FP in Fig. 22). These have been ascertained to be processes of fibroblasts (FB) which lie on the underside of the basement lamella. The fibroblasts contain large vacuoles and form a layer between the basement lamella and the underlying connective tissue (Figs. 1 and 2).

Dermo-epidermal junction:

The basal plasma membrane on the whole is quite flat and rests on a rather less dense layer (AS) of about 700 A in thickness (Fig. 15). It is underlain by a layer of denser material and shows different structures using different techniques for the preparation of the material. In osmium tetroxide fixed material and after staining with phosphotungstic acid overnight before embedding in methacrylate there is seen extensive fibrillar material spanning this layer (Fig. 23). The similarly fixed but unstained material embedded in Araldite shows no fibrillar structures. Instead flake-shaped structures are observed (Fig. 24). If, however, the methacrylate sections are stained with phosphotungstic acid there appears a sort of network within this layer (Fig. 16). The Araldite-embedded material, when stained with uranyl acetate shows definite peg-like structures on the underside of bobbins, some of which almost reach across this layer (Fig. 15). The potassium permanganate-fixed material shows flake-shaped structures and nothing else (Fig. 18). The glutaraldehyde-fixed material stained with uranyl acetate and lead hydroxide

shows this layer to be made up of electron opaque material which has a kind of patchy appearance. As a result no definite conclusion regarding the presence or absence of fibrillar material here can be reached (Figs. 1 and 25).

The layer of denser material below this adepidermal space is the dermal membrane (DM), according to the terminology suggested by Selby (1955). It is about 575 Å^o in thickness (Figs. 1, 2, 15 and 25). Towards the epidermal side it shows a more distinct border than the dermal side where sometimes thin fibrils may be seen to penetrate it (Fig. 15).

Effect of hyaluronidase on tadpole skin:

In order to gain some knowledge about the cementing substance between the two contiguous plasma membranes at desmosomes and between the plasma membrane of basal cells and dermal membrane, the skin was treated with hyaluronidase.

Observations with a stereomicroscope showed that a part of the epidermis comes off either in single cells or in groups of cells. Electron microscope studies showed that the cells separate at desmosomes (DS) because of the dissolution of the cementing substance between the two contiguous membranes (Fig. 26). Fig. 27 shows a desmosome which has just been split. It is interesting to note that the two plasma membranes are still close to each other at places other than the desmosome. It seems as if the cementing substance has given way to some tensile forces pulling the

two membranes apart at desmosomes. This shows that Selby (1955) was right in giving the name tonofibrils to the filaments, which are anchored on one side on the plasma membrane within the desmosome with their other ends penetrating into the interior of the cell.

Another equally conspicuous effect of hyaluronidase is the collapsing of the adepidermal space (Fig. 28). The plasma membrane of the basal cell does not remain separated from the dermal membrane by the adepidermal space but shows protruberances which seem to reach the dermal membrane. It is intriguing to note that instead of the cell membrane being detached by the dissolution of cementing substance under the influence of hyaluronidase it shows protruberances towards the dermal membrane. No explanation can be advanced for this at present.

DISCUSSION:

Epidermis as a protective covering:

The skin of tadpole, as is that of other fresh water vertebrates, is a flexible protective covering which can not only withstand minor physical stresses but also help in the prevention of general hydration of the animal. In both these functions the skin was thought to be assisted by a layer of cuticle which was described to border the external surface of outer epidermal cells (Weed, 1934; Weiss and Ferris, 1954b). It was also described that the cuticular layer bridges over the intercellular spaces so that an unbroken surface is

presented to the outside. During the present study, however, no cuticular layer has been observed. Instead there is present beneath the plasma membrane a layer of vesicles situated in the cortical region of the outer cells. The contents of these vesicles are mucoid in nature as identified by an empirical staining (Carleton and Drury, 1957). Histochemically these are PAS positive, stain only faintly with Alcian blue, do not stain metachromatically with toluidine blue and have been ascertained to be mucoproteins (Pearse, 1961). The contents of the vesicles when released form a layer on the outer surface of the skin. The mucus may be acting as a lubricant and helping to ward off bacteria, etc.

It is known that the layer of outer cells separates off very easily from the basal cells and Weed (1934) maintained that cuticle holds these cells together to form a layer when they come off the skin. It is not the cuticle but the otherwise firm union of these cells which holds them together. The contiguous plasma membranes are very closely apposed to each other leaving between them a uniform space about 125 ⁰ A wide. This is perhaps occupied by a cementing medium which holds them together. This union is further strengthened by a series of desmosomes present here. In this way not only the cells are held together but the inward passage of water is also prevented.

The cortical region of outer as well as basal cells is occupied by filaments. The nature of this fibrillar material though is not fully established but it resembles

the tonofibrils described in epidermal cells of mammalian skin (Porter, 1954; Selby, 1955; Odland, 1958) or the filaments in cells of the keratogenous zone of hair follicles as described by Birbeck and Mercer (1957). These filaments have been referred to as keratin by Porter (1954) and Montagna (1956), but as pointed out by Matoltsy (1958) and Kemp (1959) one should be careful in applying the term keratin because it denotes a class of structural proteins rather than a single substance. Some of the filaments from the cortex converge on the desmosomes while others form bundles (figures of Eberth) to end in bobbins. The fact that sometimes both ends of these filaments may be seen attached to the plasma membrane (Figs. 16 and 17) suggests that perhaps all filaments have both their ends connected to the plasma membrane and thus linking the various attachment points, e.g., bobbins and desmosomes. Such an arrangement according to Charles and Smiddy (1957) "results in a linkage of the entire epidermal tonofibrillar network into an elastic system adapted to taking up the distortional strains which beset the epidermis".

Basement lamella as a strengthening element:

The collagen fibrils embedded in a matrix constitute, undoubtedly, the strengthening element of the skin. Collagen fibrils are present in all vertebrate skins, connective tissue and tendons and are known to withstand great tensile forces. The orthogonal arrangement of the fibrils would have perhaps given the greatest strength against such tensions,

while still allowing the skin to stretch.

The matrix serves to keep the fibrils in place. Its chemical nature is not completely known. Though the tadpole skin itself has not been studied histochemically, the work done on skins of other vertebrates suggests that it consists of carbohydrate-protein complex, i.e. acid mucopolysaccharides (Gersh and Catchpole, 1949 and 1960). Of these hyaluronic acid forms a major component (Waine and Helwig, 1961).

The basement lamella is pervious to nutritive fluids which must pass through it to reach epidermal cells because blood vessels are conspicuous by their absence in the basement lamella as well as in the epidermis. The large intercellular spaces may be playing a role in the diffusion of nutritive fluids and metabolic products in the epidermis.

Dermo-epidermal junction:

Beneath the basal cell membrane is present a layer of less dense material, the adepidermal space (Singer and Salpeter, 1961), which is about 700 Å^o in width. This space is delimited on the underside by a layer of denser material, which will be called here dermal membrane, after Selby (1955). It is no longer disputed that the space and dermal membrane are not a part of the cell membrane, as described earlier by Weiss and Ferris (1945a), who were swayed by the controversy at the time about the structure of the cell membrane, but are rather extra-cellular structures.

However, the space and the dermal membrane together with the basal cell membrane showing local differentiations form a complex which maintains the dermo-epidermal junction. The morphology of this complex, especially that of the adepidermal space and the mechanism by which this junction is maintained, is more than of academic interest for it is a site of major involvement in many diseases of the skin (Pearson and Spargo, 1961) .

Weiss and Ferris (1954a and 1954b) were perhaps the first to study the junction with electron microscope in detail in amphibian larval skin. They described this space to be about 600 \AA in thickness and occupied by granules of uniform size about 500 \AA . They did not observe any fibrillar connections between the epidermal cells and the corium. Leeson and Threadgold (1961) described connections between the basal cell membrane and dermal membrane. Chapman and Dawson (1961) showed small quantities of granular and fibrillar material in the space and remarked that occasionally fibrils appear to span this region. Singer and Salpeter (1961) described the undersurface of bobbins as somewhat thorny and individual spines as continuous with extracellular fibrils (40-90 \AA in diameter) which traverse adepidermal space. They further went on to explain why other workers like Weiss and Ferris (1954a and 1954b) and Kemp (1959) observed granules instead of fibrils by arguing that the granules were actually sectioned fibrils. Edds and Sweeny (1962) did not observe any fibrillar material spanning

across the space rather they found some 'flake-shaped bodies'
(150-600 Å) lying in piles of three or four with their long
axes normal to the subjacent lamellar surfaces.

Selby (1955), based on her studies of mammalian material, suggested that the basal cell membrane is bound to the dermal membrane across adepidermal space with the aid of amorphous material (polysaccharides) found in this space. During the present study it has been observed that different structures are seen in adepidermal space using different techniques in the preparation of the material for electron microscopic observations.

The preparations stained over long periods with phosphotungstic acid before embedding in methacrylate show a network of fibrils, which seems to be an artifact. The sections from Araldite-embedded material and stained with uranyl acetate definitely demonstrate the presence of spines on the undersurface of bobbins. Potassium permanganate obviously does not fix anything other than membranes. The glutaraldehyde-fixed material shows this space to be occupied by a layer of material which is more electron opaque than not only the dermal membrane and matrix of basement lamella but also the cementing layer present at the desmosomes.

This differential appearance of the cementing layer not only demonstrates the varied effects of different fixatives but also suggests the lability of the material present in the adepidermal space.

Selby (1955) suggested the cementing substance to be polysaccharide in nature and perhaps similar to that present at the desmosomes. Histochemical studies have shown that the intercellular bridges stain intensely by Hale technique (Braun-Falco, 1958; Braun-Falco and Weber, 1958). The stained material covers the surface of both epidermal cells and the bridge. Braun-Falco and Weber both believed this cementing substance to be identical with that which binds the epidermis to the corium. They have further shown biochemically that major components of acid mucopolysaccharides are present in human epidermis.

Acid mucopolysaccharides have also been definitely established to form a matrix in which collagen fibrils are embedded in the corium. It has been observed in the present study that the cementing substance present in the adepidermal space is more electron opaque and is not as homogeneous as the matrix of basement lamella. There is, however, no doubt that hyaluronic acid is present because this space collapses when treated with hyaluronidase which also separates the desmosomes. The techniques used by various workers to separate the epidermis from the corium for various purposes (Skjelkvale, Nieder and Huf, 1960; Pearson and Spargo, 1961) further suggest the difference in the nature of the cementing substance of adepidermal space and of desmosomes.

In conclusion it seems reasonable to assume that on the undersurface of bobbins is present a layer of some

material, probably proteinaceous, which appears as spines. The surface of bobbins is glued on to the dermal membrane by a cementing substance probably mucoprotein.

Morphology of secretion in the outer cells:

As described earlier the cells of the outer layer secrete a mucoid substance which lubricates the outer surface of the skin and wards off infectious organisms.

The mucus vesicles form a layer beneath the outer plasma membrane and discharge their contents occasionally, which then form a layer on the outer surface. Fig. 8 shows a method by which these vesicles discharge their contents without any injury or damage to the parent cell. This mode of secretion conforms with the merocrine type according to Palay (1958).

The mucus vesicles originate near the Golgi region of the cell. Fig. 6 shows one of such vesicles which is enlarging at the expense of smaller vesicles. The latter in turn seem to originate in the nearby Golgi complex. This morphological evidence of the origin of secretory material in the Golgi region in the form of vesicles and these vesicles discharging their contents to the outside falls in line with the general scheme of synthesis and secretion well established in other secretory cells. According to this scheme macromolecules are synthesized at the surface of ribosomes associated with endoplasmic reticulum (Lowther, Green and Chapman, 1961). Then the synthesized material appears in the Golgi region (Revel and Hay, 1963; Porter

1964). Golgi cisternae have been shown to be in continuation with endoplasmic reticulum (Palay, 1958). The vesicles from the Golgi region coalesce with others to form larger ones which move to the surface of the cell, fuse with the plasma membrane, develop openings and then release their contents to the outside.

These cells also synthesize another material which is not secreted to the outside but appears within these cells as filamentous material, the keratin filaments.

Development of basement lamella:

The basement lamella, in the larval amphibian skin, consisting of an array of collagen fibrils in a uniform and complex pattern presents a unique material for the study of synthesis and secretion of macromolecules and their extracellular deposition and alignment in such a regular pattern of great complexity. The problem is not only of general interest but a fuller knowledge of the whole process is likely to be of considerable value in research on rheumatoids and other connective tissue disorders.

There is considerable evidence that the material for the synthesis of collagen fibrils is furnished by the fibroblasts (Porter, 1964) which are present on the underside of the basement lamella. The fibroblasts secrete the macromolecules not only as a merocrine secretion (Stearns 1940a and 1940b) but also as an apocrine secretion (Porter, 1964). These cells also secrete polysaccharides which form the matrix that holds the collagen fibrils (Porter, 1964).

Porter concludes that the protofibrils of collagen are formed at, and never beyond, the surface of fibroblasts and the latter also directs the arrangement of the fibrils.

The sequence of events during the embryonic development of basement lamella and its reconstruction during wound healing is essentially the same. The fibrils first appear at random and later on become rearranged into the orthogonal pattern. Weiss (1956) has discussed this problem at length and concluded that this arrangement "starts as a planar pattern, which then extends itself into a space lattice, as geometric order sweeps from the epidermal surface downwards to align the erstwhile random population of fibrous elements into the characteristic layered grid."

Not only does the rearrangement of fibrils start from the epidermal side but new layers also seem to be added from this side. This is clear in Fig. 15 which shows finer fibrils present in the uppermost layer. This observation is supported by the autoradiographic studies of Hay and Revel (1963). This laying out of new layers from the epidermal side together with the fact that some of the finer fibrils seem to come out of the dermal membrane suggests some role played by dermal membrane. The dermal membrane itself seems to be dependent for its development on the epidermal cells.

IIIb. ELECTRON MICROSCOPIC STUDIES
OF CHLORINE EFFECT ON TADPOLE SKIN

INTRODUCTION:

Bactericidal use of chlorine in water supplies is almost universal. Chlorine kills bacteria by selective injury to vital centers of the organism (Bringham, 1954), specifically by the inhibition of sulfhydryl enzymes as well as other enzymes sensitive to oxidation (Knox et al, 1948). Treated tapwater has adverse effects on other organisms also such as fish and tadpole, usually leading to their death. Freshwater organisms can generally be reared in tapwater, however, if the water is allowed to stand for about forty-eight hours after which time the free chlorine is usually reduced to insignificant proportions because of its great reactivity. The present study of structural changes brought about by tapwater chlorine in tadpole skin was initiated when drastic effects upon the epidermis of tadpole were noticed when a culture of these animals was accidentally introduced into chlorinated tapwater.

RESULTS AND CONCLUSIONS:

At the effective concentration of chlorine, about one part per million, tadpoles generally first show a lateral curling of the tip of the tail. Soon blisters appear beneath the epidermis (Fig. 29). Finally the epidermis begins to split, usually at the dorsal and ventral margins of the fin. From these rents the epidermis peels away in

sheets.

Electron microscopic studies show that first there is an increase in the intercellular spaces of the epidermis, which continues until the outer layer of epidermal cells is pushed away from the basal layer (Figs. 30-32). Either the desmosomes give way or the plasma membrane bursts. The cells of the outer layer remain joined to each other at the terminal bars to form the sheets. Occasionally the basal cells burst (Fig. 33) and release their contents so that what remains behind is the plasma membrane of the basal cells joined to the basement membrane.

Intracellularly, the cisternae of the endoplasmic reticulum swell (Figs. 30-32), Golgi membranes are disrupted, and with continued treatment spaces appear within the double nuclear envelope, mitochondria swell (Fig. 34) and keratin filaments lose their regular arrangement (Fig. 35).

These characteristic effects can be duplicated only with other oxidizing agents, e.g., potassium permanganate, hydrogen peroxide. The inhibition of the effects of chlorine by reducing agents, e.g., sodium sulphite and glutathione, confirms that the mode of action is oxidation. That it is not only the enzymes which are affected is shown by the fact that various enzyme inhibitors such as arsenicals, cyanide, iodoacetamide and fluoride, neither individually nor collectively produce the characteristic effects. Nor are the effects obtained by using a variety of enzymes and analogue substances. Table I lists substances used in

attempts to duplicate the chlorine effects.

Table I

Ammonium chloride	Ferric sulphate	Sodium citrate
Ammonium hydroxide	Ferrous chloride	Sodium chloride
Ammonium sulphate	Ferrous sulphate	Sodium hydroxide
Chloral hydrate	Formic acid	Sodium hydroxide + EDTA
Citric acid	Lithium lactate	Sodium potassium tartrate
Collagenase	Lithium chloride	Sodium sulphate
EDTA	Methane	Sulphuric acid
Ferric chloride	Pepsin	Trypsin
		Urea

The increase in the intercellular spaces and the swelling of intracellular organelles such as mitochondria, reticulum cisternae and the nuclear envelope suggest the general destruction of the barriers such as selective permeability of the cell membranes and a layer of mucus on the surface of the cells which normally protect the animal from general hydration.

Because the cells of the outer layer peel off in sheets and not as individual cells, it may be inferred that chlorine does not affect the cementing substance. It is by the increase in the pressure inside the intercellular spaces, due to imbibition of water by the fluid present there, that the outer layer of cells is pushed away till all the connections with the basal layer break.

IV. TADPOLE LATERAL-LINE ORGANS

INTRODUCTION:

The tadpole skin is a protective flexible covering which is endowed with a variety of sensory organs. The most conspicuous of these are the lateral-line organs. These are pear-shaped groups of cells, generally called neuromasts, each containing four types of cells. These are sensory, sustentacular, basal and mantle cells (Chezar, 1930). Whereas the sensory cells are concerned with sensory perception, the others are supportive and protective in function. The sensory cells at one end bear certain outgrowths, termed hairs by light microscopists, projecting into the external aquatic medium. At the other end they are in association with nerve fibers from the lateralis nerve. The stimulus, which comprises mechanical disturbances in the aquatic habitat, is received at the hairs and is transmitted to the nerve fibers as electrical impulses.

The lateral-line organs have been of great interest to many types of biologists. The great variety in structure and development of these sense organs have attracted the attention of histologists (Kingsbury, 1895; Pfuller, 1914; Johnson, 1917; Escher, 1925; Charipper, 1928; Chezar, 1930). Their functional homology and phylogenetic relationship with the labyrinth enhances the importance of results. Due to the superficial position of these organs and the easy accessibility of their nerves,

they have provided a favourable material for electrophysiological investigations (Dijkgraaf, 1956; Lowenstein, 1957). However, in spite of being a favourable material for cytological investigations, only a few electron microscopic studies have been done (Trujillo-Céno, 1961; Hama, 1962).

The present investigations include the study of fine structures of these organs. An endeavour is also made to discuss the findings in the light of other electron microscopic studies of various sense organs. It is also attempted to elucidate the mechanism involved in the conversion of mechanical stimuli, received by sensory hairs, into electrical impulses transmitted by nerve fibers.

OBSERVATIONS:

The neuromasts are arranged along definite lines on the body of the tadpole (Fig. 36). Each organ is a pear-shaped structure embedded in the epidermis, with a shallow external depression in its center (Fig. 37). It is made up of a group of cells in which four different types may be distinguished, as also discernible by light microscopy (Charipper, 1928; Chezar, 1930), mainly according to their topographic position in the organ and their function. The cells situated in the center are club-shaped and bear some outgrowths on their apical ends projecting into the shallow depression. These are sensory or receptor cells (RC). These cells never reach the basement lamella (BL). Their basal ends are in association with nerve endings (NE). Scattered amongst the receptor cells are sustentacular cells (SC), which are

quite slender in form and have neither the outgrowths at their apical ends nor nerve endings at their basal ends. At the bottom of the organ are basal cells (BC_1) which have their broad bases resting on the basement lamella. Their apices support the receptor and sustentacular cells while they themselves usually do not reach the external surface of the organ. The entire organ is covered on the sides by mantle cells (MC) which are watchglass-shaped and separate the organ from the other epidermal cells. A detailed account of each type of cell is given below.

Receptor cells:

Each neuromast contains about 5-10 club-shaped receptor cells (RC). At their exposed apical ends each cell bears outgrowths (Figs. 37-41). These fall into three categories.

1. Irregularly placed microvilli about 0.1μ in diameter (ML).
2. Stereocilia about 0.18μ in diameter (S), which arise in a regular pattern in lines, equidistant from each other. Their interior is electron opaque as is the apical region of the cell from which these arise. The electron opacity is due to the presence of a dense meshwork of filaments (F) of about $50 \overset{\circ}{\text{A}}$ in diameter (Fig. 41). Though no definite arrangement other than a close meshwork is discernible in these outgrowths, a tangential section through them shows longitudinal striations.

3. Kinocilia (K), about 0.36μ in diameter. Each shows the typical ciliary fine structure of nine paired tubules forming a ring around two in the center. The cilium is not situated symmetrically on the apex of the cell but is on one side of the entire group of outgrowths (Fig. 40).

Each of these outgrowths is covered over by plasma membrane which is continuous with that of the rest of the cell. The apical portion of the receptor cell whence the outgrowths arise is more electron opaque than the rest of the cell. This region is generally called the cuticular plate (CP). It seems to be formed by a dense meshwork of fine filaments about 50 \AA in diameter, which appear to be similar to those described for the stereocilia (S). From the underside of the cuticular plate arise tubular structures (MT) which penetrate deep into the interior of the cell (Figs. 42 and 43). The tubules, which henceforth will be called micro-tubules, have an outside diameter of about 250 \AA . Their wall is 50 \AA thick. It is not only thinner than the plasma membrane and the other intracellular membranes, but is also less electron opaque. The micro-tubules generally follow a straight course in the axial direction of the receptor cell. These seem to form bundles which penetrate deep into the cell and may even be seen reaching the basal region of the cell (Fig. 44) where the cell is in association with the nerve endings. Many mitochondria are present, arranged roughly parallel to the micro-tubules, between the apical region of the cell and

the nucleus (N), which is housed in the proximal part of the cell (Fig. 43). Endoplasmic reticulum with its associated ribosomes may also be seen. Golgi complexes (G) are quite prominent. Generally more than one may be seen in each cell. They consist of flattened sacs and round vesicles. The vesicles are about 350 Å in diameter. There are also present some intensely electron opaque spherical granules about 0.3μ in diameter. These are melanin granules which are present in epidermal cells as well.

The nucleus of the receptor cell is round with a few invaginations. The cytoplasm present in the proximal region of the cell contains various organelles, i.e. mitochondria, endoplasmic reticulum, Golgi, microtubules. It is also very rich in vesicles of about 350 Å in diameter. There are literally clusters of vesicles (Fig. 46). They seem to originate in the Golgi complexes described above.

In association with the plasma membrane of the proximal region of receptor cell are nerve endings (NE) with which it forms synapses. Each synapsis (Figs. 46 and 47) consists of the plasma membrane of receptor cell, and the plasma membrane of the nerve ending, separated from each other by a space of uniform width, the synaptic cleft (SCL). On the basis of various structures present close to the plasma membrane in the receptor cell and in the nerve ending, two types of synapses can be distinguished.

1. Beneath the receptor cell membrane are present a few vesicles. These are very close to the membrane.

The nerve ending (NE_1) contains a large number of mitochondria but very few vesicles (Fig. 46).

2. The nerve ending (NE_2) contains a large number of vesicles and a few mitochondria (Figs. 46 and 47).

Under the receptor cell membrane are sometimes present flattened sacs (SA) lined by membranes similar to the plasma membrane or intracellular membranes. Three such sacs are discernible in Fig. 47. The adjacent sacs are separated by a thicker membrane, presumably formed by the fusion of two contiguous membranes lining the sacs. The membrane immediately beneath the receptor cell membrane does not fuse with the latter and a space of uniform width about 130 Å is present here. The width of each sac is uniform but not quite the same as other sacs. The innermost sac is about 150 Å, the middle is about 220 Å and the outermost, i.e. towards the receptor cell membrane, is about 130 Å. The membranes lining the sacs show in their association ribosomes at certain places. The innermost sac seems to be in continuation with the endoplasmic reticulum.

The synaptic cleft is of uniform width, about 265 Å. The pre- as well as post-synaptic membranes in the region of synapsis do not show any structural differentiations.

The nerve endings are recognisable by the presence of vesicles of about 350 Å in diameter, and lack of any

endoplasmic reticulum or ribosomes. The mitochondria present are slightly smaller than those in receptor cells.

Sustentacular cells:

These cells (SC) are present between the receptor cells. Their cytoplasm is rich in various cytoplasmic organelles (Figs. 37, 48 and 49). The most conspicuous structures are the much-enlarged cisternae of the endoplasmic reticulum (ECE) which seem to store an amorphous substance. There are also abundant Golgi complexes with associated vesicles. These vesicles range from about 300 Å to 400 Å in diameter. Even a cursory glance gives the impression that these cells are engaged in some kind of synthesizing activity. The product of synthesis may be stored in the enlarged cisternae of the endoplasmic reticulum and the Golgi-associated vesicles, which perhaps represent two phases of the same process. The vesicles resemble in size those present in the receptor cells and the nerve endings. Occasionally an amorphous material (SP) similar to that present in the enlarged cisternae of the endoplasmic reticulum may be seen in the intercellular spaces in the organ (Fig. 50).

Basal cells:

These cells (BC) have their bases attached to the basement lamella by means of bobbins etc. similar to those described earlier in the basal epidermal cells. Their upper ends support the sustentacular cells and the receptor

cells which do not reach the basement lamella (Figs. 37 and 50). These cells resemble the sustentacular cells in the presence and distribution of various organelles. There is present endoplasmic reticulum with its enlarged cisternae containing amorphous material. Golgi complexes and vesicles similar to those in sustentacular cells and receptor cells are also present.

Mantle cells:

These cells (MC) surround the neuromast and separate it from the epidermal cells (Figs. 37 and 51). Their bases are attached to the basement lamella like the basal cells and the other end reaches the external surface of skin. Thus these cells form a wall around the entire organ. The cytoplasm resembles that of sustentacular cells and basal cells in showing enlarged cisternae of endoplasmic reticulum, Golgi complexes, and a few mitochondria.

The top of the organ is covered by extensions of epidermal cells (Fig. 37) of the outer layer, which form lips around the organ, leaving only a small area in the center uncovered through which the receptor cells project their outgrowths.

The various cells comprising the lateral-line organ are united by means of desmosomes (Fig. 37) similar to those described in the epidermal cells. The basal part of the organ contains large intercellular spaces which are often traversed by intercellular bridges and nerve fibers. The

upper part of the organ is devoid of any intercellular spaces. The contiguous plasma membranes here are closely apposed to each other and especially close to the external surface this space is reduced to a minimum. This too is perhaps occupied by a cementing substance like that in the terminal bars in other epithelial tissues.

The organs are supplied by nerve fibers from the lateral-line nerve present immediately beneath the basement lamella. A cross section of the nerve (Fig. 52) shows both types of nerve fibers, myelinated (MNF) and unmyelinated (UMF). The nerve fibers pass through the basement lamella to reach the receptor cells. The fibers (Fig. 53) which traverse the basement lamella are devoid of any myelin sheath but a Schwann sheath may be seen (SchC). Beyond the lamella the fibers are devoid of any covering. They differ from the nerve ending in having a few vesicles and a large number of mitochondria.

Fig. 54 shows a cell (NC) which seems to be moving through the basement lamella into the epidermis. It differs from the epidermal cells (EC) in lacking keratin filaments, in the size of mitochondria which are considerably smaller and the presence of various dense bodies which are less frequent in the epidermal cells. This cell is not in structural union with the epidermal cell by means of desmosomes etc. and it is accompanied by naked nerve fibers (NF).

DISCUSSION;

Structure:

The sensory cells of neuromasts have been associated with stimulus reception, whereas sustentacular, basal and mantle cells have been considered to be supportive and protective in function. Recently Hama (1962) by electron microscopic studies has described only two types of cells in the lateral-line organs of Japanese sea eel (Rhyncocymlea nystromi). In the lateral-line organs of tadpoles of Rana pipiens it has been observed that on the basis of fine structure alone, the cells other than sensory resemble each other closely and can be grouped together as supportive cells. These cells in addition to being supportive and protective in function seem to be engaged in some kind of synthesizing activity. This is suggested by the presence of ribosomes, endoplasmic reticulum with its enlarged cisternae, Golgi complexes and their associated vesicles. The enlarged cisternae of the endoplasmic reticulum seem to be the storage sites of synthesized material. Neither the site of release of this material nor its function is very clear. It seems unlikely that these cells contribute to the formation of the cupula; which will be discussed later. Though no vesicles have been seen to release their contents at any place, a material similar to that present in the cisternae has been occasionally observed in the intercellular spaces. Perhaps the material from the cisternae is released directly into those spaces. This would

imply that the contents of the intercellular spaces of neuromasts are different from those of intercellular spaces present in the epidermis in general, as no such secretory activity has been observed in the epidermal cells. This is further supported by the fact that the mantle cells seem to form a complete covering around the organ and separate the intercellular spaces of the organ from those in the epidermis. The conclusion that the contents of the intercellular spaces of receptor organ are different was also arrived at by Springle (1962) on the basis of electrophysiological studies.

Lateral-line organs have been described to bear cupulae. Gorner (1961) described the cupulae in Xenopus to be made up of transparent jelly-like material. Although several investigators have sought and not been able to observe cupulae in Xenopus (Murray, 1955; Dijkgraaf, 1956) Gorner insists that they are nevertheless present. He claims that the cupulae are very delicate structures and are easily lost in routine histological techniques. Even in the fresh state on the living animal they may be crushed or brushed off in handling. Further that even if they are present they are difficult to see due to their transparency. In the lateral-line organs of living Rana pipiens tadpoles they have not been observed even with phase-contrast microscope, in spite of great care in handling.

The cupular substance is generally supposed to be secreted by supportive cells although such a role has also

been attributed to sensory cells and intercellular substance (Denny, 1936 and 1937). According to Gorner (1961) it is mainly produced by the mantle cells. He claims that these cells differ from other supportive cells not only in the shape of their nuclei but also in staining reactions. No cytological difference which could explain the difference in staining has, however, been observed and although all the supportive cells are engaged in the synthesis of some material, there is no evidence that this material passes toward the outer surface where the cupula is supposed to be. Regarding its origin from the intercellular substance, though, as mentioned earlier, the material seems to be secreted into the intercellular spaces, the space seems to be sealed off from the exterior.

Each sensory cell bears on its apical surface certain outgrowths which seem to be concerned with perception of stimulus. These consist of a large number of stereocilia and a single kinocilium. They have generally been termed 'hairs' by light microscopists and electrophysiologists (Lowenstein, 1957; Dijkgraaf, 1956). It may be pointed out that these structures constitute living parts of sensory cells and are not secretory products or other dead material. The outgrowths are homologous with similar outgrowths on the internal and external hair cells in the organ of Corti (Friedmann, 1962) and sensory hairs in the gravity receptors in utricle and saccule (Spaendlin, 1964). The hair cells in the organ of Corti have undergone extensive histochemical

investigations by Vinnikov and Titova (1963). These authors have shown that the hairs react positively to tests for acetylcholinestrerase, alkaline glycerol phosphatase and acid glycerol phosphatase. These workers claim that the hairs are extremely chemosensitive antennae protruding into the endolymph.

The apical region of the receptor cell, from which the various outgrowths arise, is quite electron opaque and because of its lack of any structure it has been generally called a cuticular plate. It has been observed that this region and the stereocilia are full of a meshwork of filaments.

From the underside of the cuticular plate arise a large number of microtubules. These penetrate deeply into the receptor cell. The microtubules resemble in their morphology those present in the cilia. Similar tubular structures have also been described in mitotic spindles where these are concerned with the movement of chromosomes (Harris, 1962; Roth and Daniels, 1962), in maturing spermatids which shows a collar extending from the margin of the nucleus (Burgos and Fawcett, 1956). They also seem to be similar to neurofilaments present in the nerve fibers where there are some indications of cytoplasmic movement. Their fine morphology has been studied by Ledbetter and Porter (1963 and 1964) and they have been shown to be formed by a ring-like arrangement of subunits. Pease (1963) and Andre and Thiery (1963) believed the subunits to number nine or ten filaments, while Ledbetter

and Porter (1964) demonstrated thirteen. The filaments which form a meshwork in the cuticular plate and the stereocilia seem to resemble these subunits. This is lent credence by the fact that the microtubules take their origin from this meshwork quite insensibly. Ledbetter and Porter (1963 and 1964) have concluded that the microtubules are concerned with the movement of their surrounding cytoplasmic ground substance. In the receptor cells they may also be concerned with some sort of cytoplasmic movement which perhaps is initiated by the movement of stereocilia and kinocilium under the impact of mechanical stimuli.

Sensory cells contain a large number of vesicles of about 300-500 ^o A diameter. These are very numerous in the perinuclear region especially toward the basal end of the cell where it is in association with the nerve endings. Morphological evidence suggests their origin from the Golgi complex. The vesicles resemble in shape, size and the electron opacity of their contents the synaptic vesicles present in other sensory cells such as taste buds (Trujillo-Céno^z, 1957; DeLorenzo, 1958; Murray and Murray, 1960), inner and outer hair cells of organ of Corti (Smith and Sjöstrand, 1961) sensory cells in lateral-line organs of other animals (Trujillo-Céno^z, 1961; Hama, 1962) and nerve endings (Palay and Palade, 1954; de Robertis and Bennett, 1955). The first suggestion that the vesicles might contain a chemical transmitter came from de Robertis and Bennett (1955). Del Castillo and Katz (1955 and 1956) saw in the vesicles a

possible morphological counterpart of quantitized release of transmitter substance detected by Fatt and Katz (1952) by electrophysiological studies. Many attempts have been made to isolate this substance and identify it chemically (Gray and Whittaker, 1960 and 1962; Whittaker, 1961) and it is generally agreed that the transmitter substance exists in these vesicles as bound acetylcholine. It may be pointed out, however, that the evidence for the identification of acetylcholine is mainly biological.

Nerve endings:

As mentioned earlier the synapses in the lateral-line organs are of two types. These resemble the type 1 and type 2 described by Smith and Sjöstrand (1961) in connection with the external hair cells of the organ of Corti. It appears that whereas type 1 is a synapsis between the receptor cell and the afferent nerve fiber, i.e. concerned with the passage of electrical impulses from the sensory cell to the nerve fibers, type 2 is between receptor cell and the efferent fiber, i.e. is concerned with the conduction of messages in the opposite direction. It may be either inhibiting or sensitising. In the organ of Corti the type 2 ending may be in association with the Rasmussen's efferent olivo-cochlear bundle, though such an association still remains to be demonstrated electrophysiologically (Friedmann, 1962). The presence of any efferent fibers in the lateral-line organs also as yet remains to be demonstrated by the electrophysiologists. A system of extra

membranes enclosing cavities is sometimes seen below the receptor cell membrane in the type 2 synapsis. These were also noted by Hama (1961) in the lateral-line organs of the Japanese eel. Though Smith and Sjostrand (1961) suggested that these synapses are efferent in nature, they did not suggest the origin and function of these extra membranes. It has been found that such a complex is much better developed in the synapses of lateral-line organs being studied and there are definite indications of their origin from the endoplasmic reticulum. The innermost sac seems to be in direct communication with the endoplasmic reticulum and ribosome-like structures are seen at various places on the membranes lining the cavities. The cavities perhaps help in disposing of the chemical transmitter released by the efferent nerve endings.

Ontogeny of the cells in the lateral-line organs:

It has been definitely established by the works of Stone (1922, 1931, and 1937) that the entire lateral-line system, both sense organs and nerves, is developed from ectodermal placodes on the head of the early amphibian embryo. These placodes move in various directions under the two-layered ectoderm. On their way they drop off groups of cells which eventually differentiate to form both sensory and supporting cells of the lateral-line organs.

The migrating cell described on page 42 (Fig. 54) is perhaps one of such placode cells which is making its way into the epidermis where it will differentiate into either a

sensory or a supportive cell. The accompanying nerves further support this view.

Neuromast cells, sensory as well as supportive, have an origin similar to neurons providing fibers of the lateralis nerve. The presence of microtubules in receptor cells should not be surprising as similar structures, the neurofilaments, have been described in the neurons.

Receptor cells as biological transducers:

Sense organs present a great variety of structure. The simplest of these consist of naked dendritic endings such as free nerve endings in the skins of tadpoles and frogs. These perceive the stimuli directly. Others have special cells, the receptor cells, interposed in between the external environment and the dendritic endings. Here the stimulus activates the receptor cell which acts as a biological transducer. The input is some function of mechanical energy and the output is electrical in nature, the receptor potential. The taste buds and lateral-line organs belong in this category. There are others which have accessory structures which perhaps modify, in some way, the stimulus before it reaches the dendritic ending. The pacinian corpuscles in mammals belong here.

The portion of the receptor cell in the lateral-line organ which is concerned with the perception of stimulus comprises the stereocilia and kinocilia. These outgrowths have been shown, in some cases, to be covered over by cupulae, while in others cupulae are lacking. The bending of these

outgrowths in a particular plane is essential to stimulate the sense organs (Dijkgraaf, 1963). In the resting condition when the outgrowths are straight, the nerve fibers supplying the receptor cells show a spontaneous activity, the basic impulse frequency. On shearing these outgrowths in one direction there is an increase in the impulse frequency while shearing in the opposite direction has a reverse effect (Dijkgraaf, 1963).

The mechanism involved in the conversion of the mechanical stimulus into electrical impulses is not very well understood. Davis (1961) is of the opinion that the stimulus generates a graded electric charge, the receptor potential, in the receptor cell which either electrically or by release of some chemical transmitter substance induces a generator potential in the nerve endings. This in turn initiates the electrical impulses in nerve fibers.

Katz (1950) discussed the various ways by which the mechanical disturbance can initiate an electrical change across the membrane. According to Inmann (1962) the most likely of these is that as a result of mechanical disturbances there is a change in the permeability of the receptor cell membrane. This conclusion is based on the properties of the cell membrane alone and does not take into consideration the entire receptor cell, of which the membrane is just a part. Again no satisfactory explanation has been so far provided for the way in which the mechanical deformation increases the permeability sufficient enough to give rise to receptor potential.

Vinnikov and Titova (1963) have expressed the view, which seems quite logical, that the receptor potential is nothing else than an indication of the internal state of the receptor cell, where various metabolic reactions are initiated by the mechanical stimulus. They have demonstrated the presence of acetylcholinesterase, acid glycol phosphatase and alkaline glycol phosphatase in the hairs of hair cells of organ of Corti. They postulate that the hairs are exceedingly chemosensitive antennae which are depolarizable by the action of acetylcholinesterase on acetylcholine present in the endolymph under the impact of shearing waves. This energy is sufficient to start a chain of metabolic reactions of which the receptor potential is only an indication. Perhaps a similar mechanism is in operation in the lateral-line organs though it seems very doubtful if there is any acetylcholine present around the outgrowths from the receptor cells. There has not been seen any indication of any secretion being released where the cupula is supposed to be. Maybe a similar method using a different enzymatic system is in play. It is also possible that the mechanical energy, responsible for the bending of the various outgrowths initiates the metabolic reactions directly without the intervention of some chemical in the medium where these outgrowths float.

The receptor cells as mentioned earlier contain and synthesize the synaptic vesicles which perhaps contain the chemical transmitter substance. This suggests the induction

of generator potential in the dendritic ending by the release of the chemical transmitter which induces the necessary permeability changes there. Such a method of chemical transmission has been shown to occur at neuromuscular junctions and at various synapses (Fatt, 1954; McLennan, 1963; Eccles, 1964). The only evidence that chemical transmitter substance, especially acetylcholine, is not involved in the initiation of the impulses at the receptor organs comes from the studies of such sense organs as pacinian corpuscles or stretch receptors. As mentioned in the beginning these mechano-receptors differ histologically from the lateral-line organs. In addition, the various mechanoreceptors have undergone extreme specializations and the adoption of different methods for the initiation of impulses is not out of order.

The receptor cells show a bidirectional response. The bending of the outgrowths in one direction increases, and its bending in the opposite direction decreases the basic impulse frequency. This change in the frequency may be explained in terms of the quantity of chemical transmitter that reaches the membrane of the nerve ending at the synapsis. Perhaps some quantity of such substances reaches the membrane all the time and is responsible for the basic impulse frequency. An increase in this amount will increase the basic impulse frequency and a decrease will have an opposite effect. The quantity can be controlled by changes in the permeability of the receptor cell membrane or a change in the

rate of movement of the synaptic vesicles towards this membrane.

The microtubules, which take their origin quite near the outgrowths and end in the proximal region of the receptor cell quite close to the synapses, have been shown to be concerned in some way with the movement of cytoplasm. They might act in receptor cells to cause a movement of the cytoplasm towards the synapses which will not only increase the movement of the synaptic vesicles towards the synapses but will also increase the pressure in this region, which in turn will stretch the receptor cell membrane. It is known that a stretching of cell membrane increases its permeability. In this way, perhaps, the microtubules play an important part in the increase of the basic impulse frequency. A movement of the cytoplasm in the opposite direction will have a reverse effect. This mechanism not only explains the initiation of the impulses but also the bidirectional response of the receptor cells. The cytoplasmic movement by the microtubules is perhaps initiated by the bending of the outgrowths directly.

V. STRUCTURE OF TADPOLE MELANOPHORES

INTRODUCTION:

The chromatophores play an important role in the survival of many forms of life. Amphibians have been described (Schmidt, 1920) to have various types of chromatophores which in different combinations produce the varied colors present in these animals. Melanophores give a brown or black color. Xanthophores produce yellow and erythrophores produce red color. There are also guanophores which give the animal a white color by refraction and reflection. The animals produce varied color patterns by changes in these chromatophores depending on the environmental conditions.

It was Mathews (1931) who definitely established that there is a static boundary to melanophores of Fundulus and that the pigment shifts within the cell boundary, as opposed to the earlier conception of amoeboid movement of melanophores. Herrick (1933) confirmed these observations on frog tadpole (Rana pipiens).

Ballowitz (1914) had earlier described intra-cellular tubes in melanophores and considered that the movement of pigment granules within them was caused by peristaltic contraction and relaxation of the tube walls. Behre (1935), on the basis of his study of living material, confirmed these observations.

Shanes and Nigrelli (1941), using polarized light described birefringent material intimately associated with Fundulus melanophores. They described strands of this material

interconnecting the melanophores. The material was said to show Brownian movement and changes in birefringence, both of which seemed to correlate with the degree of dispersion and aggregation of melanin.

The only electron microscopical study on melanophores of cold-blooded vertebrates is by Falk and Rhodin (1957) on Fundulus. The structure of melanophores in frog tadpoles completely differs from what was described by these authors. This study is thus aimed mainly at the correction of various incongruities in the literature regarding the structure of these peculiar cells.

OBSERVATIONS:

Epidermal melanophores:

These cells lie in the intercellular spaces present between the epidermal cells. These are slender and branched (Fig. 55). The branching is extensive in certain regions, such as the back of the tadpole, while in the tail the melanophores are less branched. The thickest region of the cell, which is almost in the middle, contains the nucleus (N).

The plasma membrane often appears to be discontinuous, perhaps owing to the effect of fixatives. It does not show any structural union with the surrounding epidermal cells in the form of desmosomes (Figs. 56 and 57). The cells are thus lying free in the epidermal intercellular spaces.

The melanophores contain a large number of intensely opaque melanin granules. They appear circular to oblong in

shape, about 0.3μ to 0.5μ in size. Each melanin granule is surrounded by a membrane which resembles in thickness and electron opacity other intracellular membranes and the plasma membrane. In the branches, the melanin granules lie close to the plasma membrane and other cellular organelles generally lie internal to these granules. Through the core of each branch pass bundles of fine filaments (KF) which extend to the distal end of the branch (Figs. 56-59). Each filament is about 60 \AA in diameter (Fig. 60) and appears to be similar to the keratin filaments described earlier in the epidermal cells. Along with these filaments in Fig. 59 is seen a double walled structure (MT). It has an outer diameter of about 250 \AA and appears to be similar to microtubules described earlier in the receptor cells of lateral-line organs (see page 37).

Though there is very little cytoplasm present around the nucleus, there is visible a Golgi complex (G) in this region (Fig. 61). It shows its usual structure.

Sometimes in the perinuclear region of the cell (Figs. 62 and 63) and sometimes in the branches (Fig. 64) a large number of membrane-bound structures are visible. These may be divided into two groups on the basis of their internal structure.

1. Vesicular structures of varying sizes showing a dense amorphous or lamellated interior but no vacuoles or electron opaque structures (DB).

These resemble the dense bodies described by

Novikoff and Essner (1960) in liver cells.

2. Comparatively large structures which show vacuoles and intensely electron opaque bodies of various sizes and shapes. These are the lysosomes (LY). Some of them are also internally lamellated but lamellae are thicker than those present in the dense bodies (compare Figs. 65 and 66).

Figs. 62-67 show the varied shapes and structures of lysosomes and dense bodies observed in these cells. Occasionally a large area of denser cytoplasm is observed in which are present a large number of melanin granules (Fig. 67). This is perhaps a large lysosome.

Dermal melanophores:

These cells are situated under the basement lamella and are embedded in the connective tissue of the dermis. They are flat structures, the thickest region being in the center which accommodates the nucleus (N). The peripheral region becomes progressively thinner and sometimes shows branches (Fig. 68).

The plasma membrane is quite smooth. The dermal melanophore does not show any structural union with neighboring cells. The outer surface of the plasma membrane is coated with a layer of dense material (Figs. 69-70). The plasma membrane with this layer of dense material seems to be similar to that of other cells embedded in the connective tissue, such as muscle fibers.

There are a large number of melanin granules which resemble, in shape and size, the melanin granules of epidermal melanophores. Each granule here too is surrounded by a membrane.

The various organelles such as mitochondria and the Golgi complex are not very conspicuous. Lysosomes, dense bodies and keratin filaments which abound in the epidermal melanophores have not been observed.

DISCUSSION:

As mentioned earlier, Ballowitz (1914) and Behre (1935) had described intracellular canals in the melanophores and considered that the movement of pigment granules was due to the peristaltic contraction and relaxation of the tube walls. No such canals or tubes have been observed in the present study. Falk and Rhodin (1957), who studied the fine structure of dermal melanophores of Fundulus, have not described anything like the canals or tubes. These authors, however, described a membrane about 80 Å thick, dividing the cytoplasm into two regions. The inner region contains, in addition to nucleus and cellular organelles, the melanin granules. The outer region, which is delimited by a membrane about 1200 Å thick, is exclusively occupied by filaments. The membrane delimiting the outer region has on its outside a layer of dense material similar to that present in other cells embedded in dermal connective tissue. These authors believe that under the influence of the filaments present in the outer region, which they

considered contractile, the inner region is stretched. This corresponds to the dispersed state of melanin granules. With the relaxation of filaments, the inner region contracts and the melanophore appears contracted. During the present examination of dermal and epidermal melanophores of frog tadpole no division of the cytoplasm into two regions has been observed. The melanin granules and the various organelles are dispersed throughout the melanophore in its expanded state. It is possible that because the dermal melanophores are embedded in dermal connective tissue which has filaments in it, Falk and Rhodin (1957) might have erroneously considered this surrounding part as the outer region of the cytoplasm of melanophore. Fig. 70 shows a part of the dermal melanophore embedded in connective tissue containing filamentous structures which could be mistaken for a part of the melanophore.

Although both epidermal and dermal melanophores originate from the neural crest (Du Shane, 1943; Horstadius, 1950; Willier, 1953) they seem to have acquired certain differences between themselves during their final differentiation. The two types of melanophores differ in the presence of keratin filaments in epidermal and absence in dermal melanophores. They further differ in the presence of a large number of lysosomes and dense bodies in the epidermal melanophores and their complete absence in the dermal melanophores. Again they differ in the presence of a layer of dense material on the outside of the plasma

membrane of dermal melanophore while it is absent in the epidermal melanophores.

Lysosomes and dense bodies have been studied by a number of workers (De Duve et al, 1955; Novikoff and Essner, 1960; Essner and Novikoff, 1961; Holt and Hicks, 1961; Novikoff, 1961; De Duve, 1963). These membrane-bounded structures, especially lysosomes have been shown to have a number of hydrolytic enzymes. There is compelling evidence (De Duve, 1963) that lysosomal hydrolases are the main agents in the catabolism of regressing tissues. The tadpoles epidermal cells in early stages (11 mm.) have large number of melanin granules. In the later stages (15 mm.), however, only a few such granules are present, and these cells also possess very few lysosomes. Further, lysosomes in the epidermal melanophores possess a large number of melanin granules in them. These facts suggest that the melanin granules of epidermal cells are catabolised in the melanophores. This would involve a cytotrine transfer of melanin granules from epidermal cells into melanophores present in the epidermis. A similar cytotrine transfer of melanin granules has been described from melanocyte into keratinocytes of hair cortex (Birbeck, Mercer and Barnicot, 1956; Barnicot and Birbeck, 1958; Birbeck and Barnicot, 1959; Birbeck, 1962).

Birbeck (1962) studied the mechanism of melanin granule synthesis in the melanocytes of human epidermis. He believed that Golgi-derived vesicles become larger, either

by growth or accretion, to form the premelanosomes which have a complex internal structure. Inside the outer limiting membrane a system of sheets develops and melanin is subsequently deposited upon these sheets. During the study of epidermal melanophores as well as dermal melanophores of tadpoles nothing comparable to premelanosomes has been observed. Probably there is no melanogenous activity at this stage of growth of tadpole (12-24 mm.).

Charles and Ingram (1959) described the melanin granules as being present directly in the cytoplasm, i.e., not surrounded by a membrane in the melanocytes or lying within a vacuole, i.e., surrounded by a membrane in a phagocyte in mammalian skin. This led them to postulate that whether melanin granules are found inside a vacuole or directly in the cytoplasm may be the result of changed environment of the melanocyte, of its physiological deterioration. However, Birbeck's pictures (Birbeck, 1962) show the mature melanin granules in the epidermal melanocytes surrounded by a membrane. I have also, during this study, observed melanin granules, in both types of melanophores, epidermal as well as dermal, always surrounded by a membrane.

Ultrastructure and function of microtubules have been discussed earlier (page 46) and it seems that these structures are concerned with intracellular movement of cytoplasm. A similar mechanism can easily be visualized for the movement of melanin granules in the melanophores.

A microtubule-like structure has been observed in Fig. 59 and it seems possible that the movement of melanin granules is effected by the microtubules.

VI. SUMMARY

The skin of frog tadpole (Rana pipiens) consists of an epidermis underlain by basement lamella. The epidermis comprises two layers of cells, the outer cells and basal cells. The outer cells are actively engaged in the secretion of mucus. The secretory product forms a layer on the outside of the skin and probably helps in warding off infectious organisms. The morphology of secretion resembles the general secretory cycle well-established in other secretory cells. The cells of the basal layer, in addition to being a part of the flexible protective covering (epidermis as a whole) are specialized for the union of the epidermis with underlying basement lamella.

The adepidermal space, which separates the epidermis from the dermal membrane, is occupied by a cementing substance. Different structures appear in the space with different fixatives employed for the preparation of the material for electron microscopy. The nature of the cementing substance present in the adepidermal space and in the desmosomes, on the basis of studies of hyaluronidase treated skin, seems to be mucoprotein.

The basement lamella consisting of an array of collagen fibrils embedded in a ground substance constitutes a strengthening element of the skin.

The epidermis peels off in sheets when it is treated with chlorinated tapwater. Electron microscopic studies have shown that at first there is an increase in

the intercellular spaces and then either the desmosomes give way or cells burst. The cells of the outer layer, on account of their firm union, remain attached together to peel off in sheets. What remains behind is the basement lamella and the attached plasma membrane of the basal cell. Intracellularly, mitochondria and cisternae of the endoplasmic reticulum swell, Golgi membranes are disrupted, keratin filaments lose their regular arrangement and spaces appear in the double nuclear envelope. On the basis of these observations it has been concluded that the barriers which normally protect the animal from general hydration are affected. The mode of action of chlorine has been found to be oxidation of not only the sulfhydryl enzymes but also other cell constituents liable to oxidation.

The functional morphology of lateral-line organs has been studied. The four types of cells, receptor, sustentacular, basal and mantle, which are distinguishable on the basis of their position, shape, nuclei and their function, can be grouped under two headings on the basis of their fine structure. The receptor cells bear microvilli, stereocilia, and kinocilia. The single kinocilium of each receptor cell is situated on one side of all the outgrowths of one cell and is towards the center of the outgrowths of all receptor cells. The receptor cells show tubular structures of about 250 ⁰ A in diameter in addition to various other organelles. These microtubules seem to play an important role in the initiation of nerve impulses at the synapsis.

The synaptic vesicles, which perhaps contain the transmitter substance, originate in the Golgi complex. Two types of nerve endings have been distinguished on the basis of their fine structure. These are afferent and efferent nerve endings. The receptor cell shows an arrangement of membrane-lined sacs in association with efferent nerve endings. The supportive cells on the basis of their fine structure, appear to be engaged in some kind of synthesizing activity and the secretory product is released into the intercellular space in the basal region of the lateral-line organ.

The epidermal melanophores have been found to lie free in the epidermal intercellular spaces. There is no structural union with the surrounding epidermal cells. The melanin granules are always surrounded by a membrane. In addition to organelles such as mitochondria and Golgi complex these cells contain lysosomes, dense bodies and keratin filaments. There seems to be no melanogenous activity in these cells. It seems that these cells, in addition to being melanin effector cells, are also concerned with the catabolism of melanin granules, which are perhaps collected from neighbouring epidermal cells by cytotrine transfer.

The dermal melanophores are embedded in the dermal connective tissue. Their plasma membrane resembles that of other cells embedded in the connective tissue in having a coating of dense material on its outside. These cells

differ from the epidermal melanophores in lacking lysosomes, dense bodies and keratin filaments.

In the epidermal melanophores a tubular structure of about 250 ⁰ A has been observed. This seems to be similar to microtubules associated with cytoplasmic movement, which could be responsible for the movement of melanin granules.

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VIII. EXPLANATION OF FIGURES

Figs. 3, 29, 36, 55 and 68 are light micrographs. The rest are all electron micrographs. From the original 35 mm. negatives, prints were made, labelled and then copied. Consequently there is some loss of resolution in these micrographs.

All magnifications on electron micrographs are based on the carbon grating replica (28,800 lines per inch) available from Ernest F. Fullam, Inc., Schenectady, N. Y.

Fig. 1. A vertical section of the skin of the tadpole.

Note the two cell thick epidermis underlain by basement lamella (BL) .

BC: basal cell; ER: endoplasmic reticulum;

FE: figure of Eberth; G: Golgi complex;

IS: intercellular space; MV: mucus vesicle;

OC: outer cell; OS: outside.

Glutaraldehyde-osmium tetroxide fixation,

Araldite embedding and lead hydroxide

staining. X 22,000.

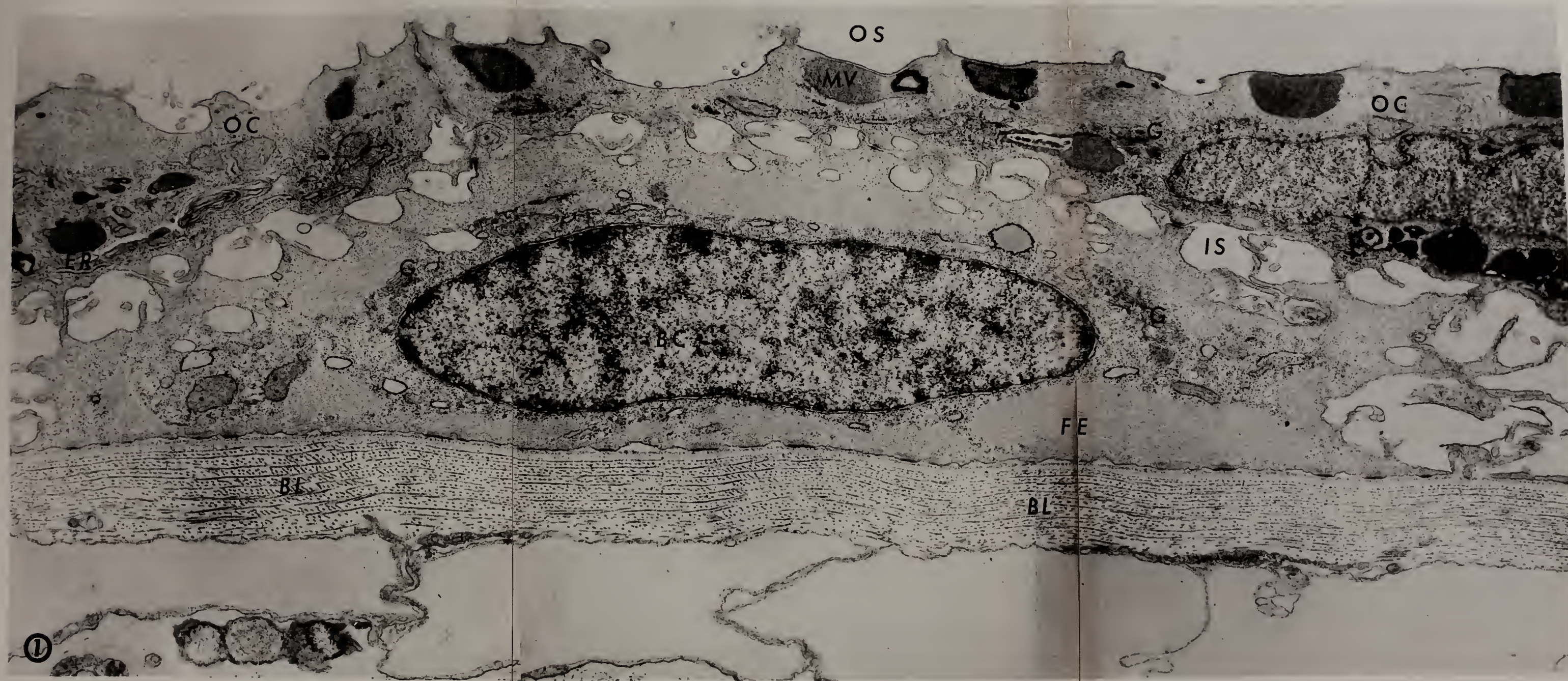


Fig. 2. Similar to Fig. 1 but a different fixative, osmium tetroxide, has been used. Note the difference in the general preservation of the cell contents.

BC: basal cell; BL: basement lamella; ER: endoplasmic reticulum; FB: fibroblast; FE: figure of Eberth; G: Golgi complex; IS: intercellular space; M: mitochondrion; MV: mucus vesicle; N: nucleus; OC: outer cell; OS: outside.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 9,000.

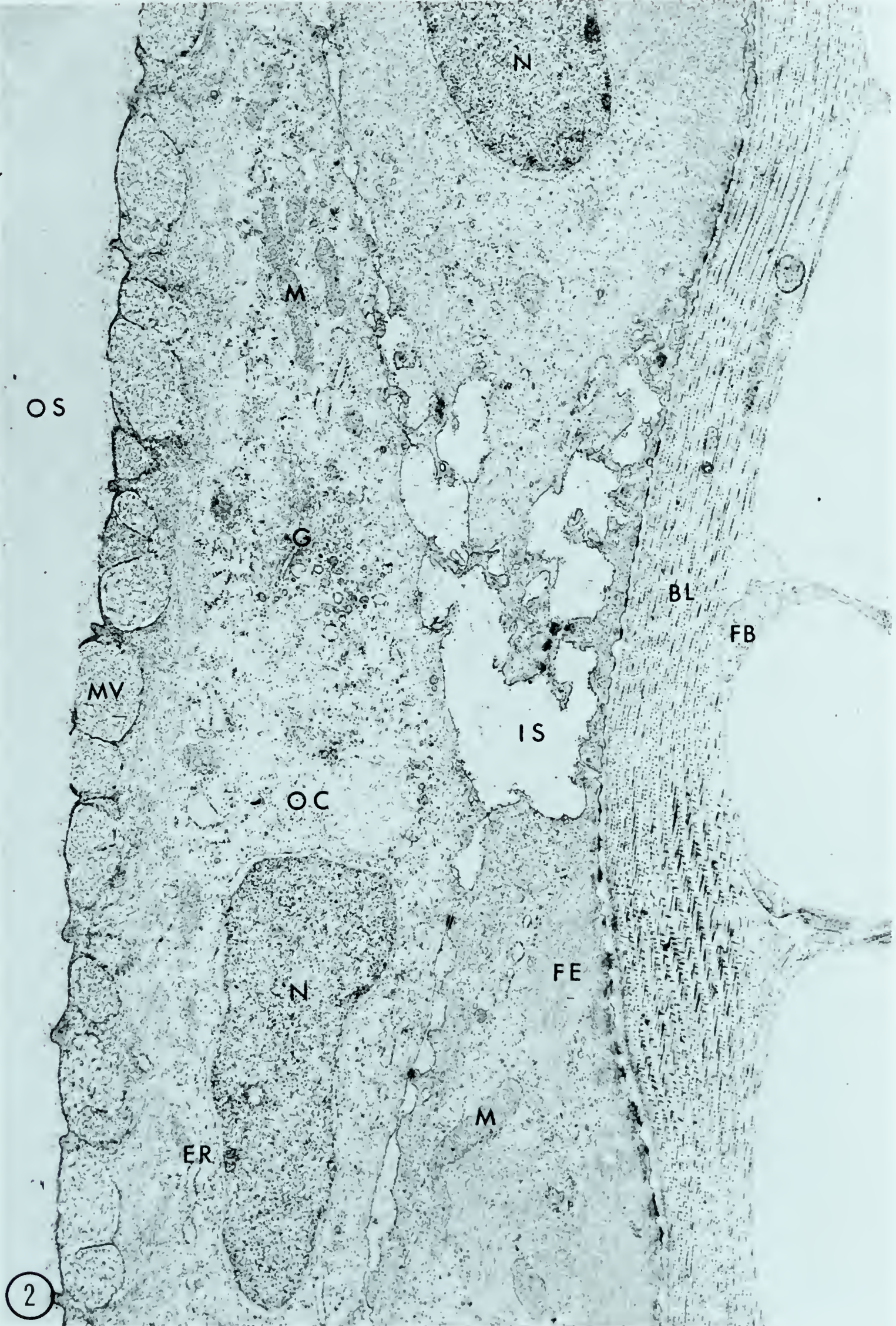


Fig. 3. A phasecontrast micrograph of the surface of the outer cells. Note the polygonal shape of these cells and the dark mucus vesicles (MV). X 2,700.

Fig. 4. A vertical section through the mucus vesicle (MV). Note the keratin filaments forming a meshwork around the vesicle.
Osmium tetroxide fixation, Araldite embedding and phosphotungstic acid (PTA) staining.
X 25,000.

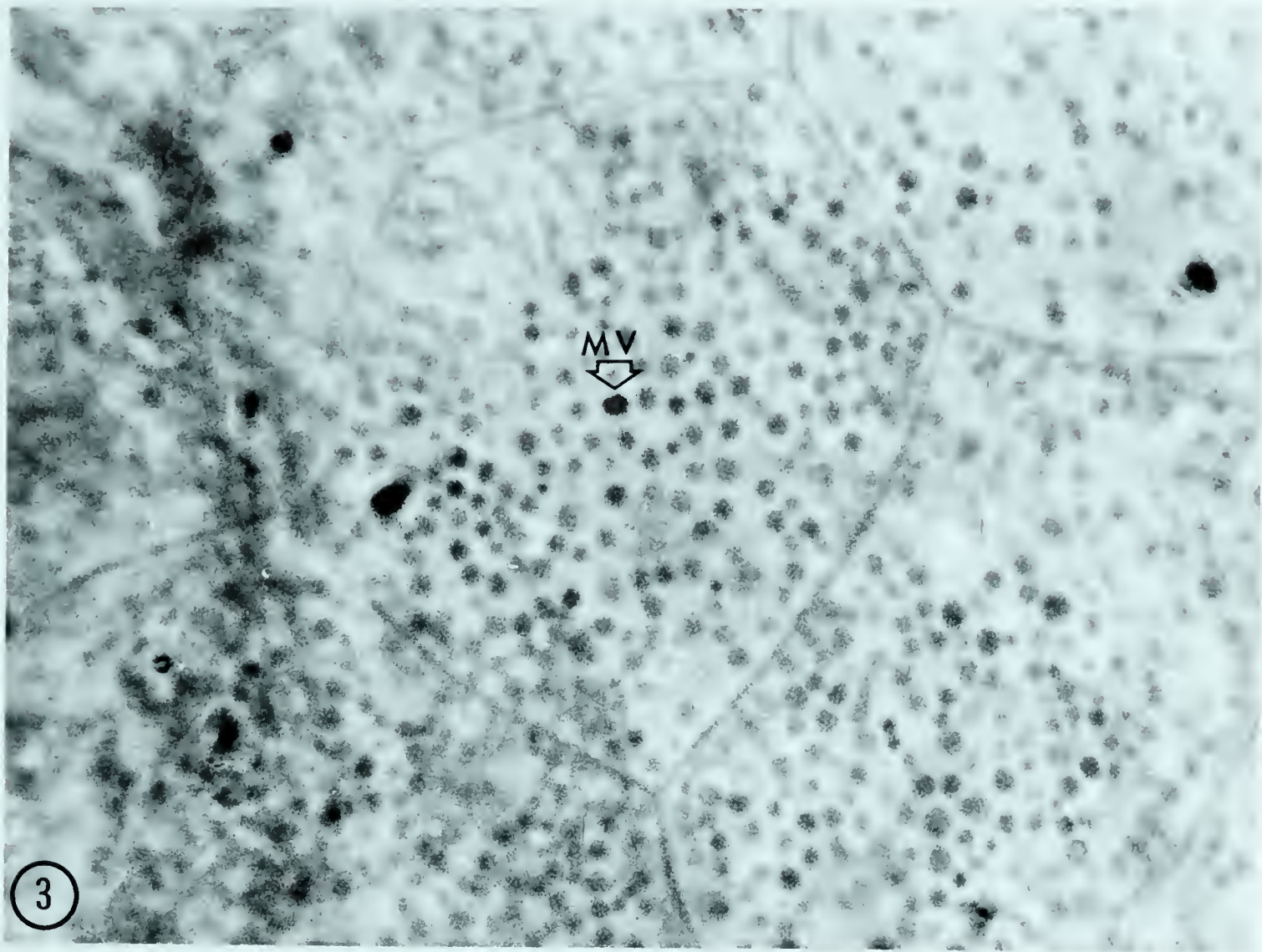


Fig. 5. A section parallel to the surface of the skin passing through the mucus vesicles (MV) . These membrane-bound structures are surrounded by a network of keratin filaments (KF) .

Osmium tetroxide fixation, Araldite embedding and PTA staining. X 18,200.

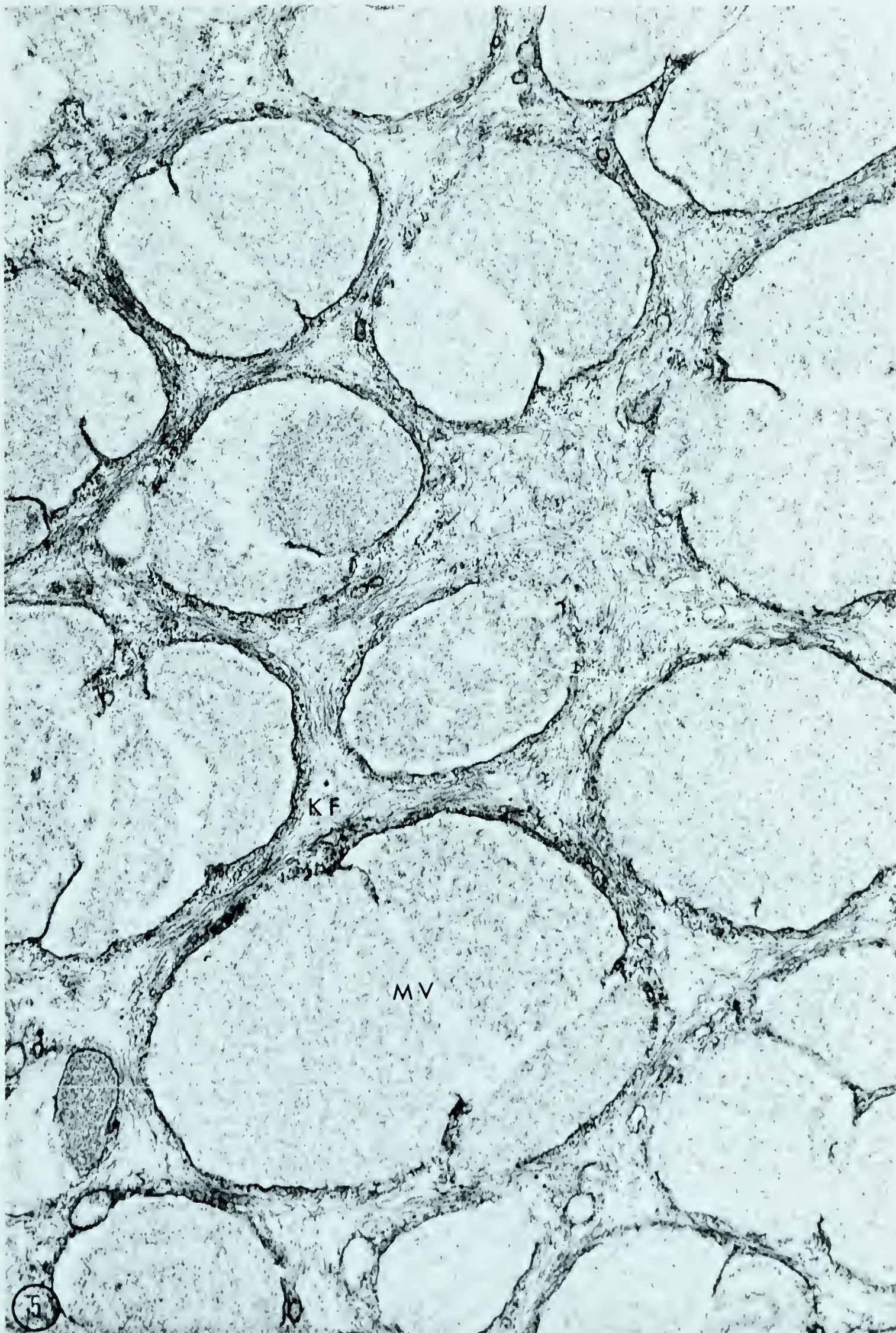


Fig. 6. A vertical section through an outer cell showing mucus vesicles (MV) . In the center of the picture is a vesicle which is growing at the expense of smaller vesicles. These in turn seem to arise in the nearby Golgi region (G) .

Osmium tetroxide fixation, methacrylate embedding and uranyl staining. X 19,000.

Fig. 7. A vertical section through the outer region of an outer cell showing mitochondria (M) forming a layer beneath the mucus vesicles (MV) .

Osmium tetroxide fixation, methacrylate embedding and uranyl acetate staining.
X 21,000.

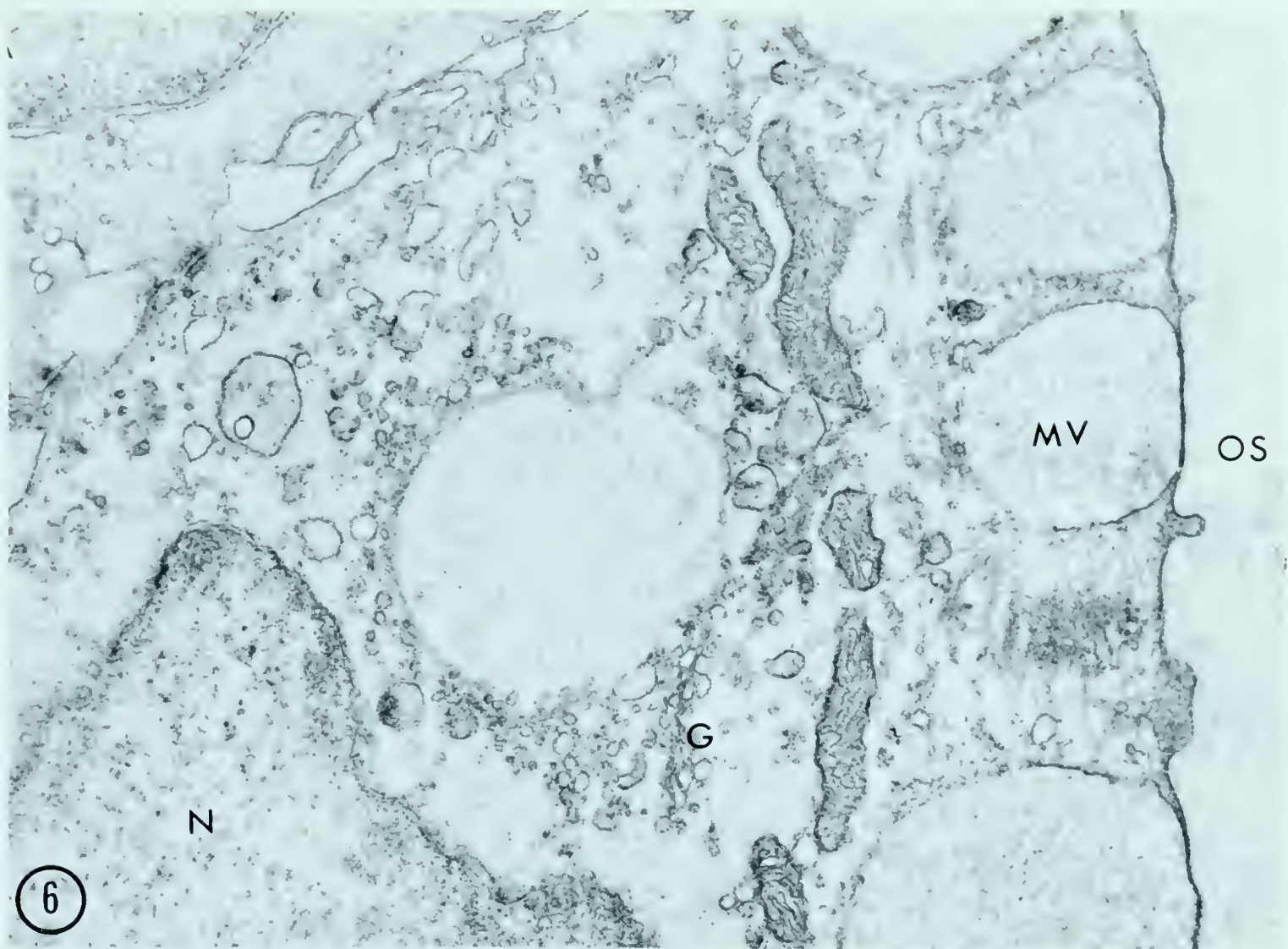


Fig. 8. A vertical section through the outer region of an outer cell. A mucus vesicle is seen releasing its secretory product to the outside. Note that the membrane of the vesicle has become continuous with the plasma membrane. Osmium tetroxide fixation, Araldite embedding and PTA staining. X 16,800.

Fig. 9. A vertical section through the site of the union of two contiguous outer cells. Note the protuberance projecting towards the outside (OS) and the close apposition of the two contiguous membranes deeper to this protuberance. This union is further reinforced by the desmosomes (DS). A distinct difference in the thickness of the plasma membrane exposed to the outside and that not thus exposed is visible. Glutaraldehyde-osmium tetroxide fixation, Araldite embedding and lead hydroxide staining. X 37,500.

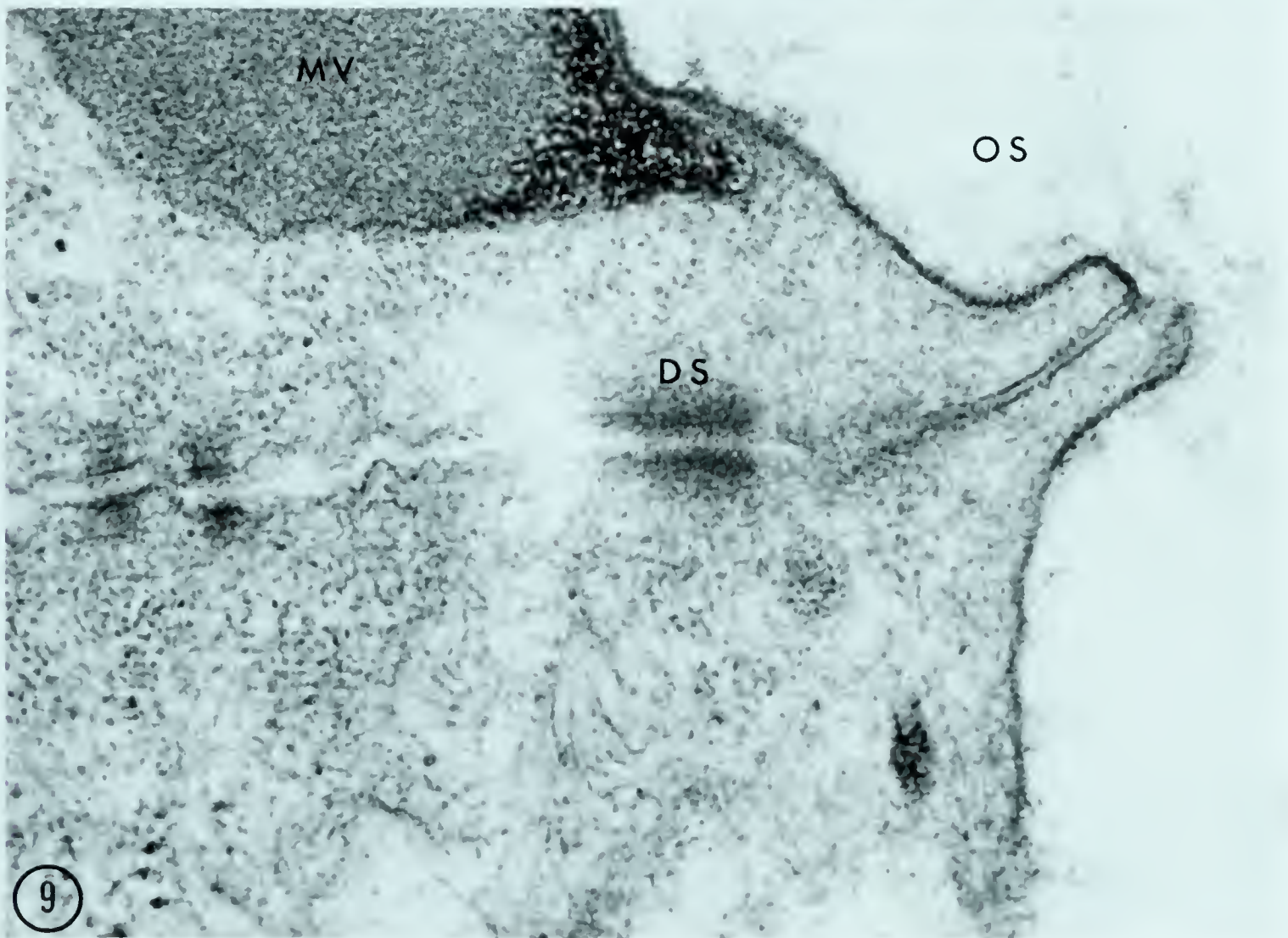
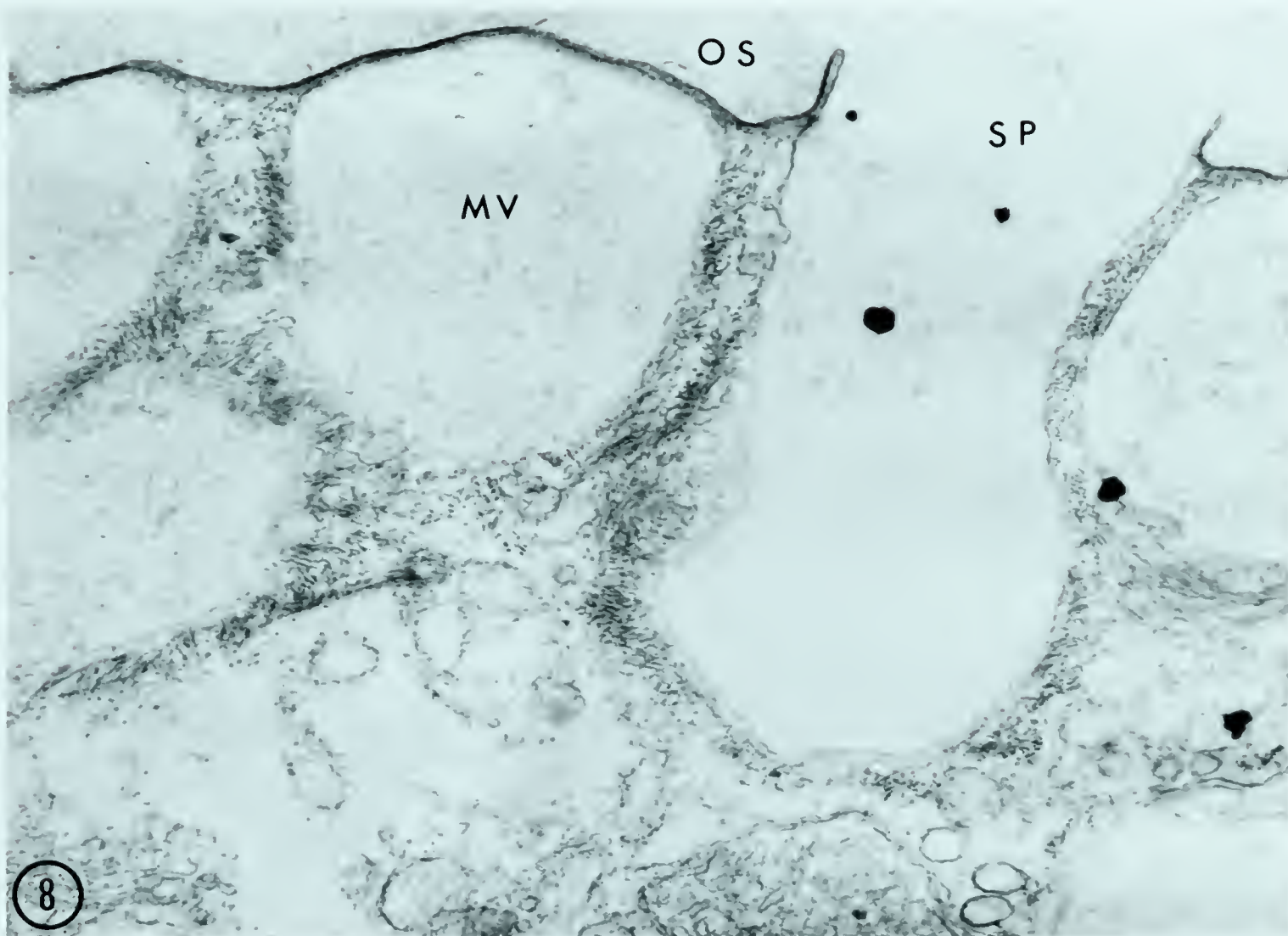


Fig. 10. A section grazing the surface of the outer cells. Note the microrugae which are present all over the surface of the skin especially around the mucus vesicles (MV). Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 13,200.

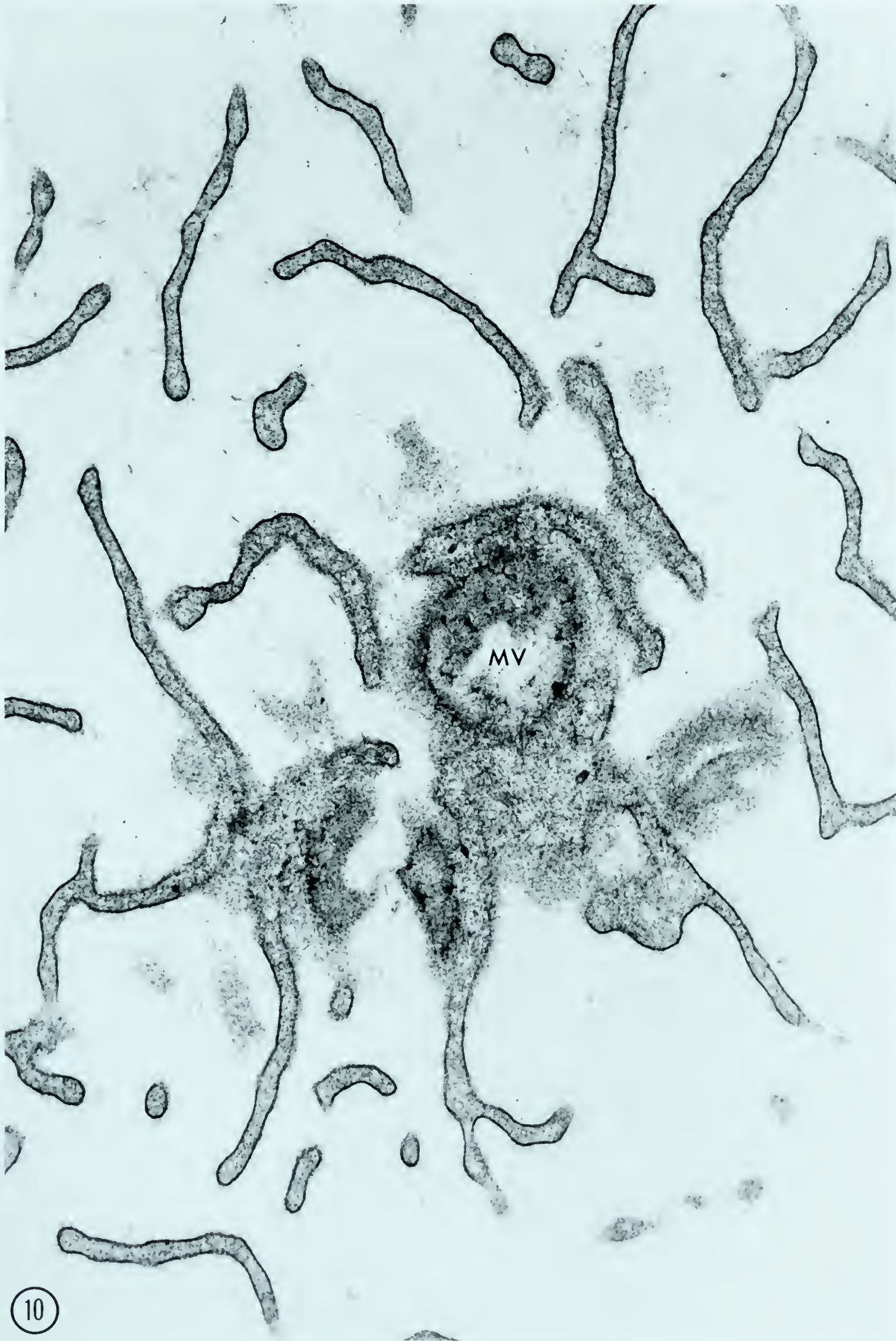


Fig. 11. A tangential section through an outer cell showing the various organelles.

ER: endoplasmic reticulum; G: Golgi complex;
KF: keratin filaments; LY: lysosome; MV:
mucus vesicle; V: Golgi-derived vesicle;
N: nucleus.

Osmium tetroxide fixation, Araldite
embedding and lead hydroxide staining.

X 21,000.



Fig. 12. A vertical section through the epidermis showing that the contents of the Golgi cisternae and vesicles (G) in the outer cells appear electron opaque in Glutaraldehyde-osmium tetroxide fixed material.

N: nucleus of outer cell; IS: intercellular space; KF: keratin filaments in the figure of Eberth in the basal cell.

Glutaraldehyde-osmium tetroxide fixation, Araldite embedding, lead hydroxide staining. X 36,400.

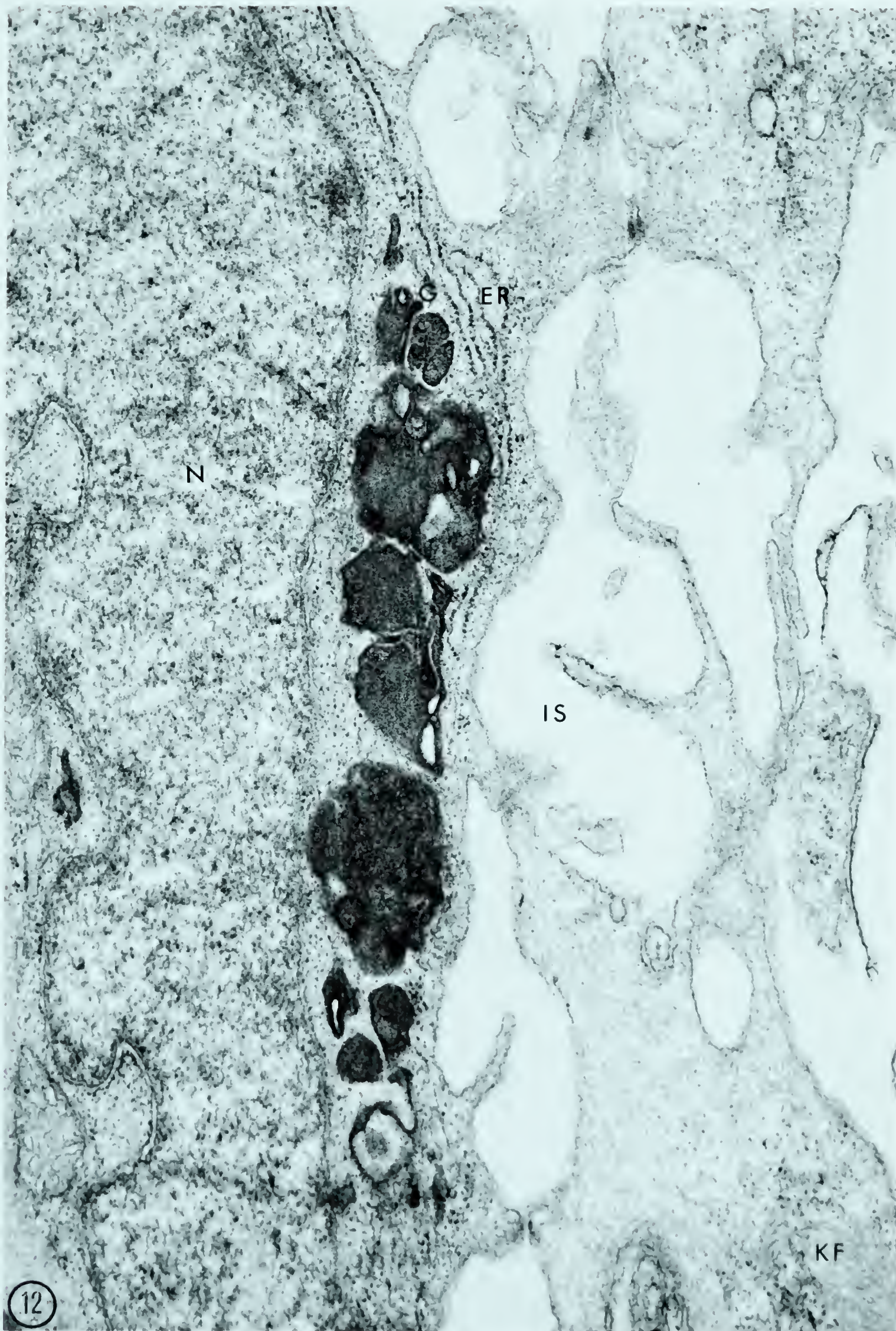


Fig. 13. A horizontal section through the site of union of two outer cells. Note a row of desmosomes (DS).

CS: cementing substance.

Osmium tetroxide fixation, Araldite embedding, uranyl staining. X 26,500.

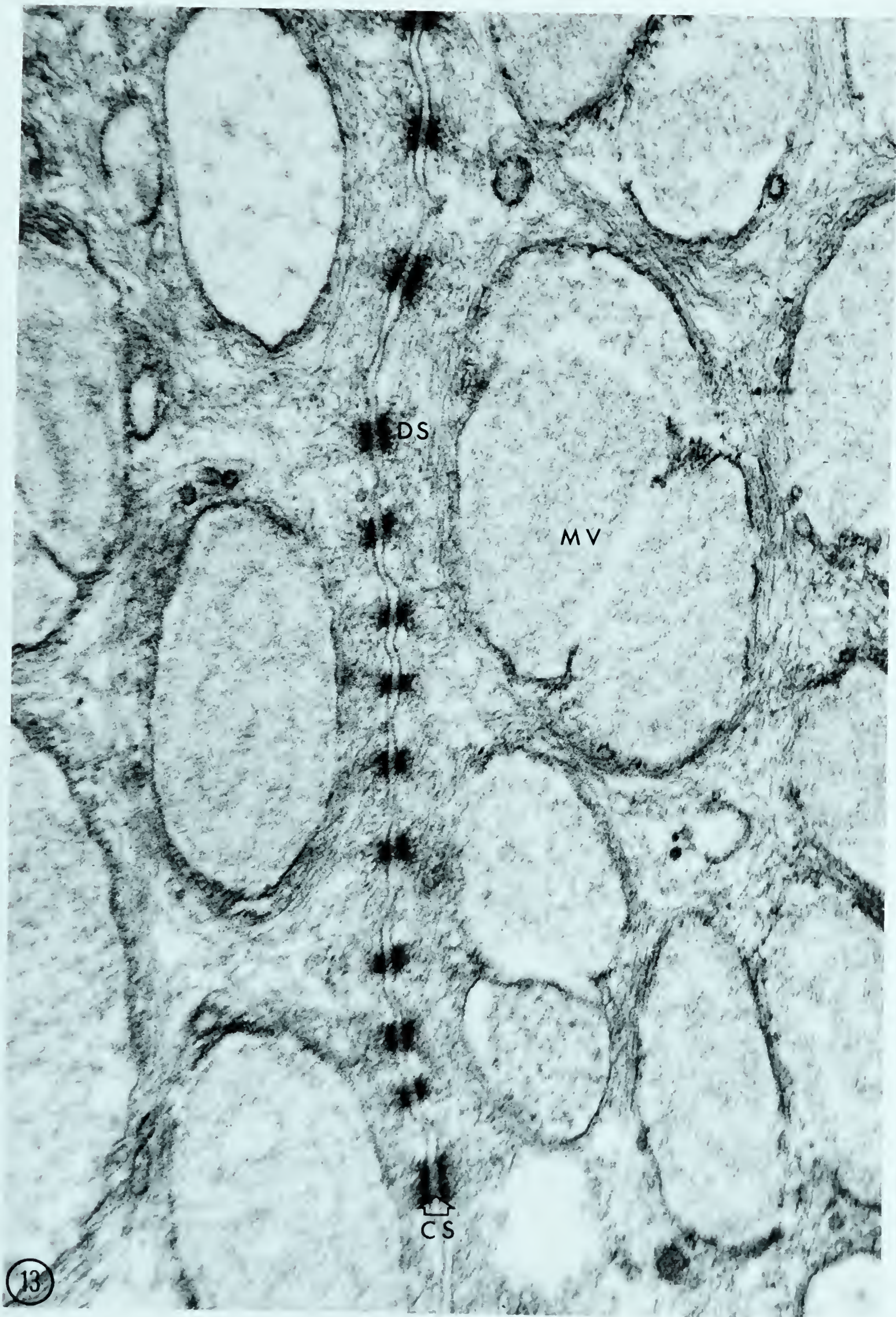


Fig. 14. A horizontal section through the basal cells. These cells are joined together by inter-cellular bridges (IB). Each such bridge has a desmosome (DS). The nucleus (N) of basal cell is lobulated and the cytoplasm shows figures of Eberth (FE) in addition to the various organelles.

M: mitochondrion; ER: endoplasmic reticulum. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 11,550.

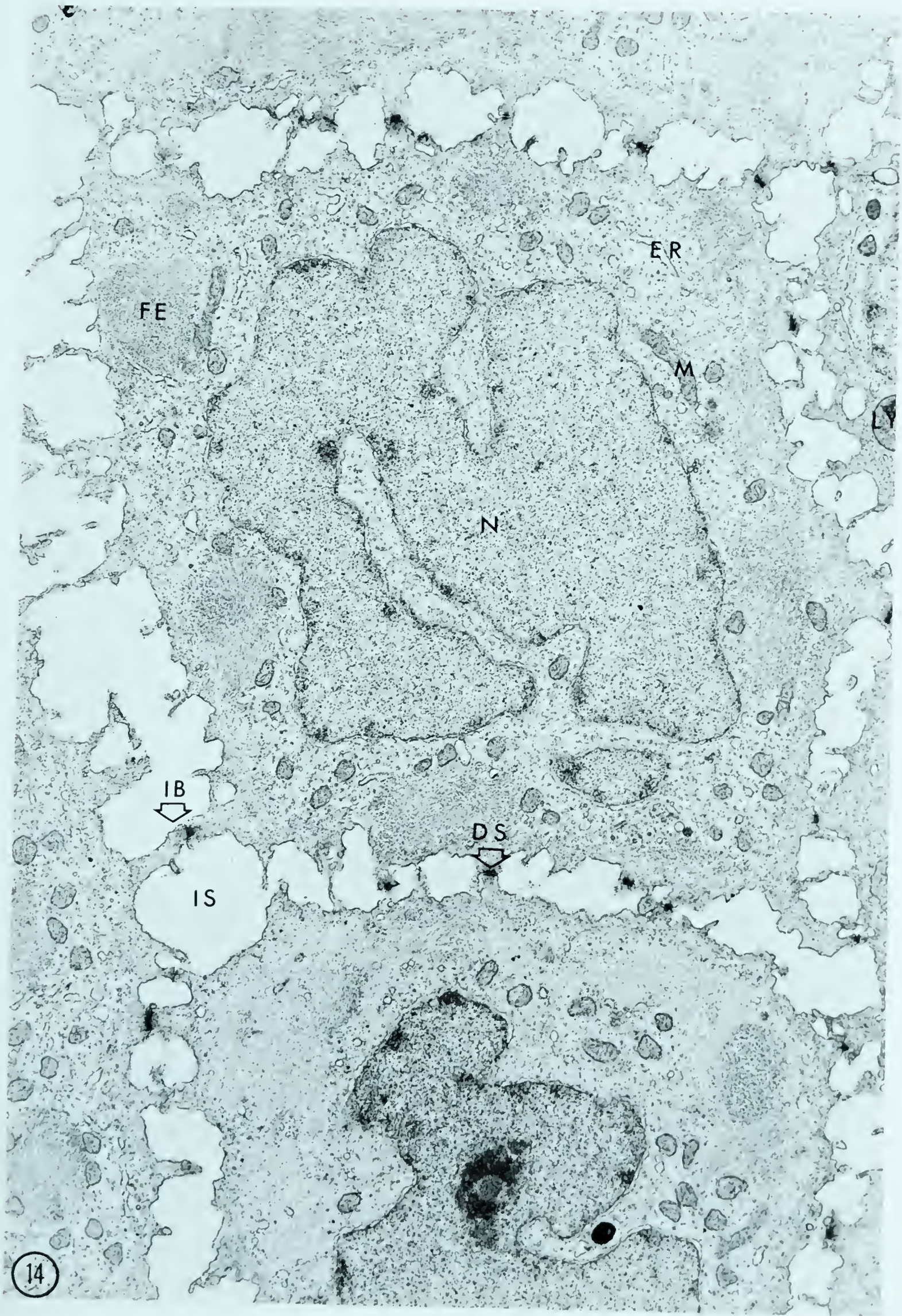


Fig. 15. A vertical section through the dermo-epidermal junction showing a part of the basal cell and a part of the basement lamella (BL). Note basal cell membrane (BCM) separated from the dermal membrane (DM) by adepidermal space (AS). Each bobbin (B) consists of two thickenings (D_1 and D_2) separated by a lighter area. Also note that the inner thickening (D_2) is made up by the keratin filaments (KF). Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 25,000.

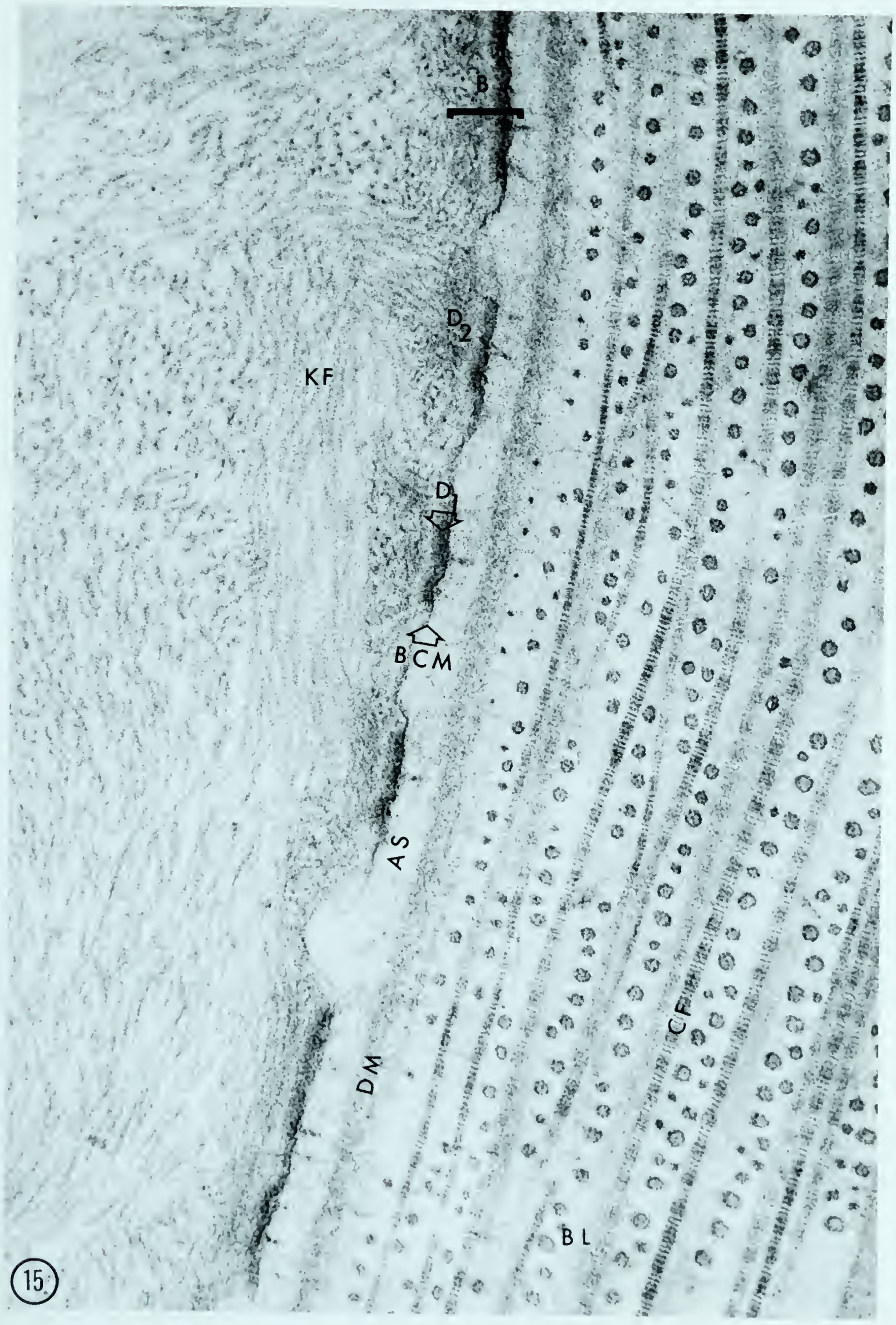


Fig. 16. A vertical section through the basal region of the basal cell showing the dermo-epidermal junction and a part of the basement lamella. Note the keratin filaments (KF) forming a loop with both their ends joined on to the basal cell membrane at adjacent bobbins. The ad-epidermal space (AS) shows a sort of network.

BL: basement lamella; DM: dermal membrane.

Osmium tetroxide fixation, methacrylate embedding and PTA staining. X 55,000.

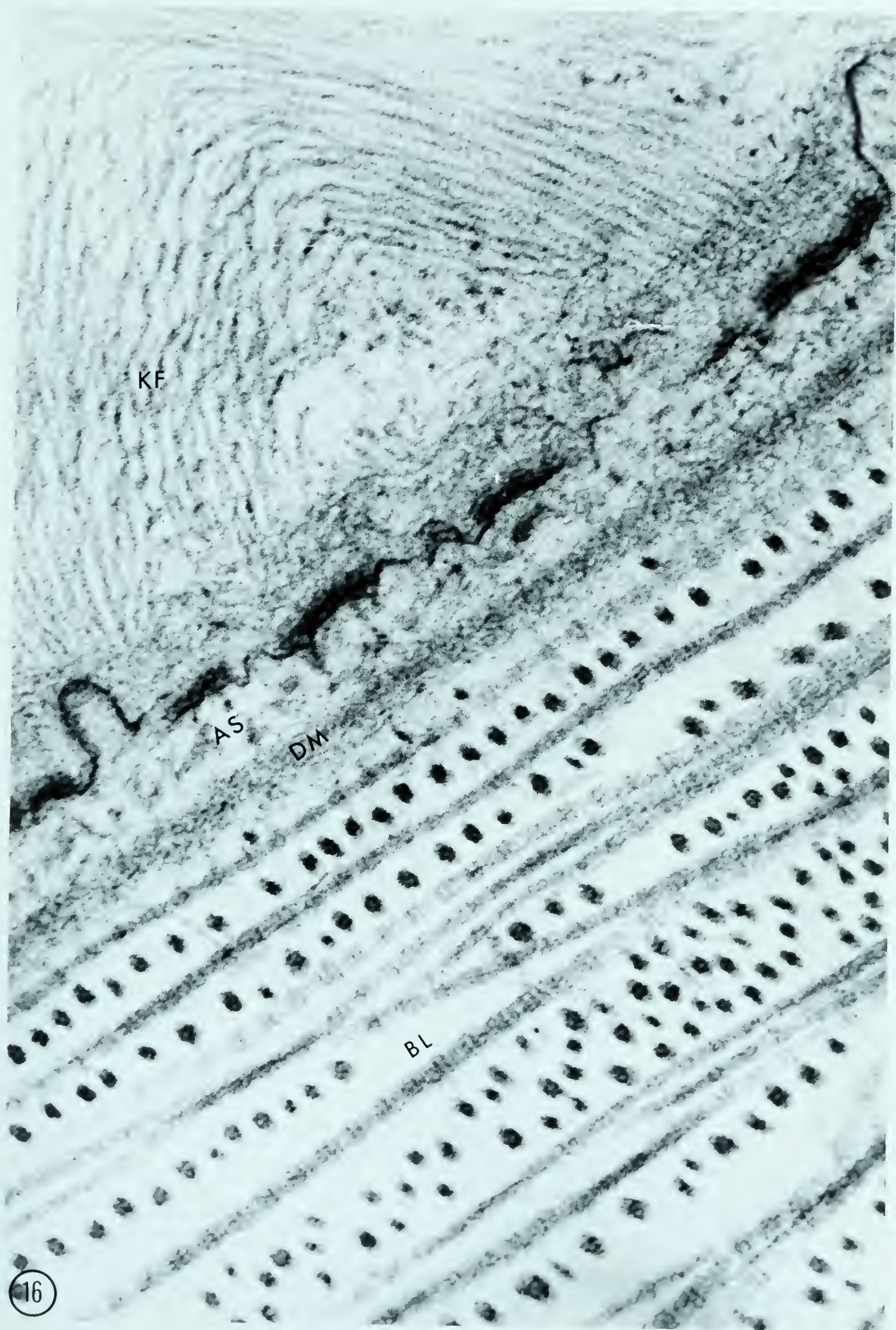


Fig. 17. A vertical section through the basal region of basal cell. Note some of the keratin filaments (KF) have one of their ends attached to the basal cell membrane (BCM) and the other at desmosome (DS). Also note the splitting apart of the desmosome by dissolution of the cementing substance. This material was treated for one hour with hyaluronidase before fixation. BL: basement lamella; M: mitochondrion; N: nucleus. Osmium tetroxide fixation, Araldite embedding, uranyl acetate staining. X 28,000.

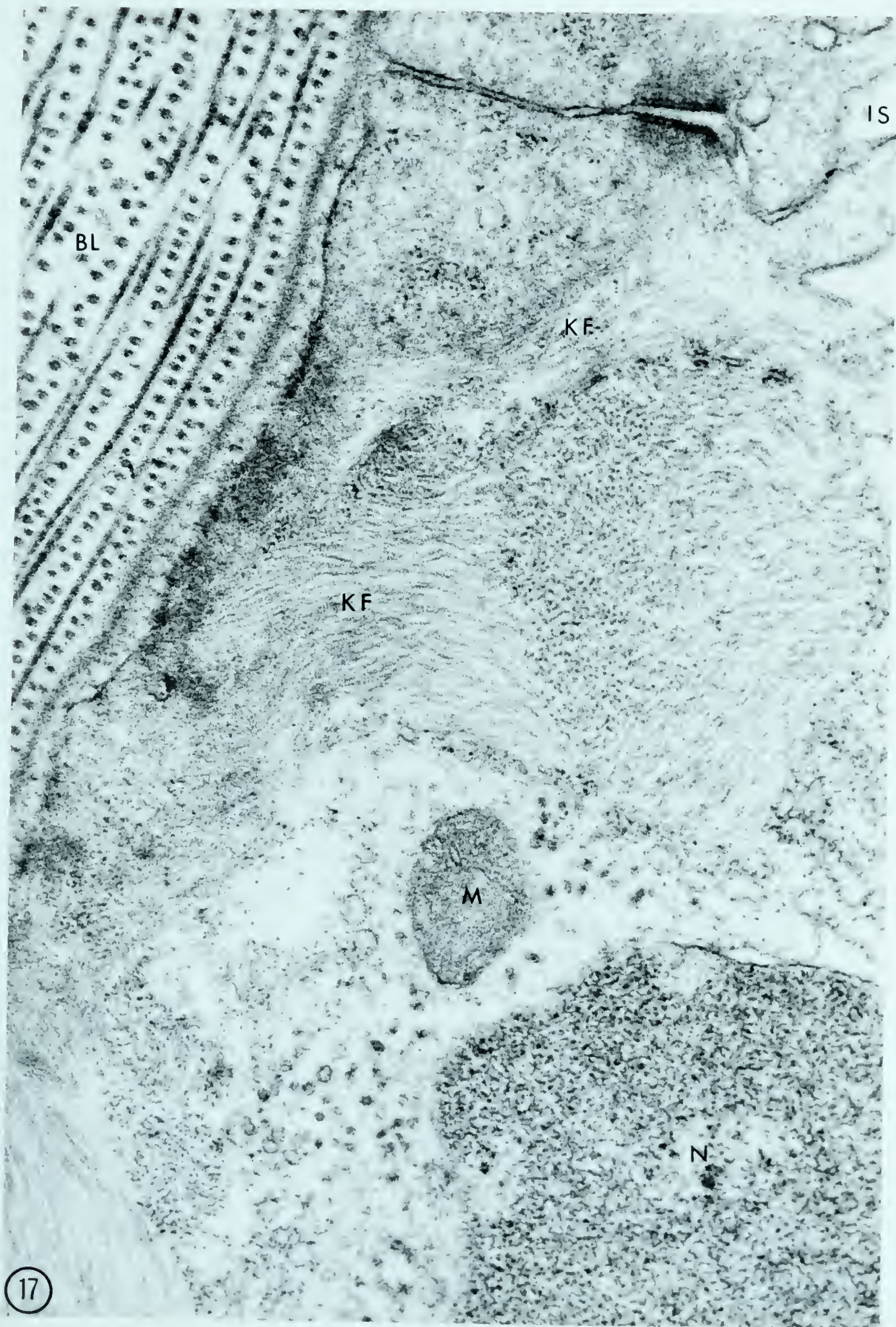


Fig. 18. A vertical section through the basal region of the basal cell. Note the dense amorphous material in place of keratin filaments. Dermal membrane (DM) and basement lamella (BL) are not distinct. The adepidermal space (AS) is occupied by flake-shaped structures and basal cell membrane (BSM) is smooth and does not show any structural differentiations. ER: Ergastoplasm; N: nucleus. KMnO_4 fixation, Araldite embedding and unstained. X 24,000.

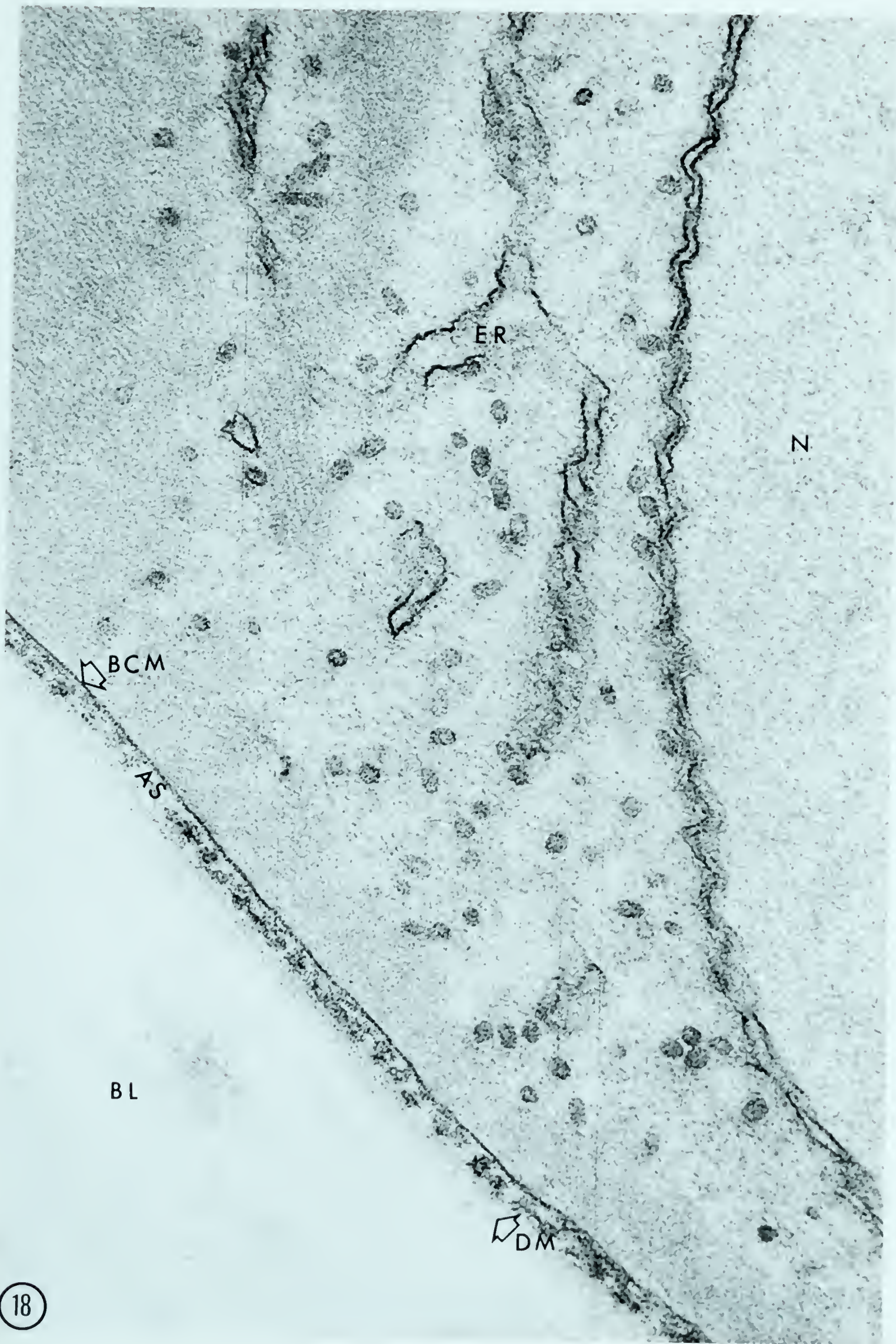


Fig. 19. A vertical section through the basal cell showing keratin filaments (KF), endoplasmic reticulum and free polysomes. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 35,000.

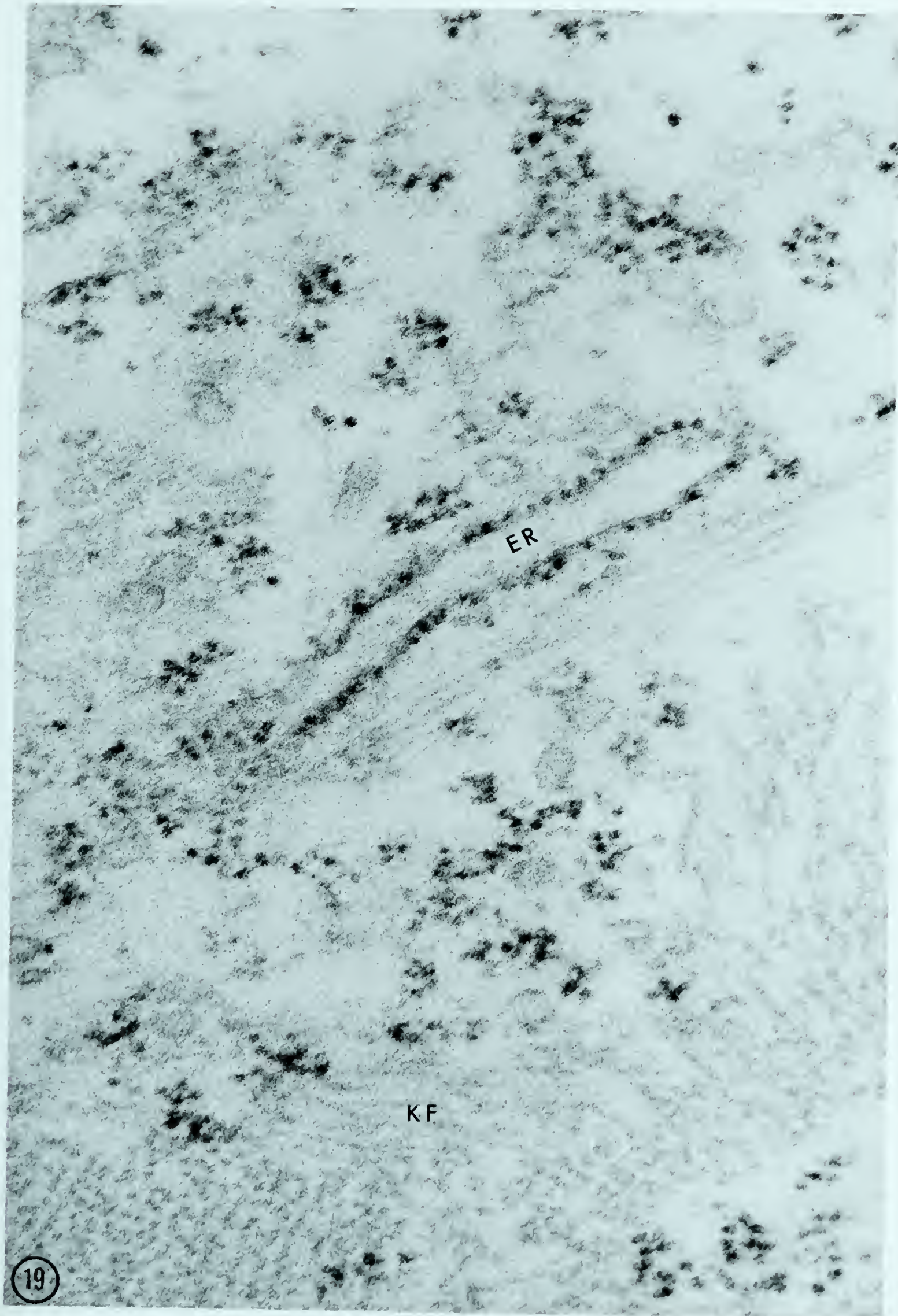


Fig. 20. A horizontal section through the basement lamella showing orthogonal arrangement of collagen fibrils.
Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 31,000.

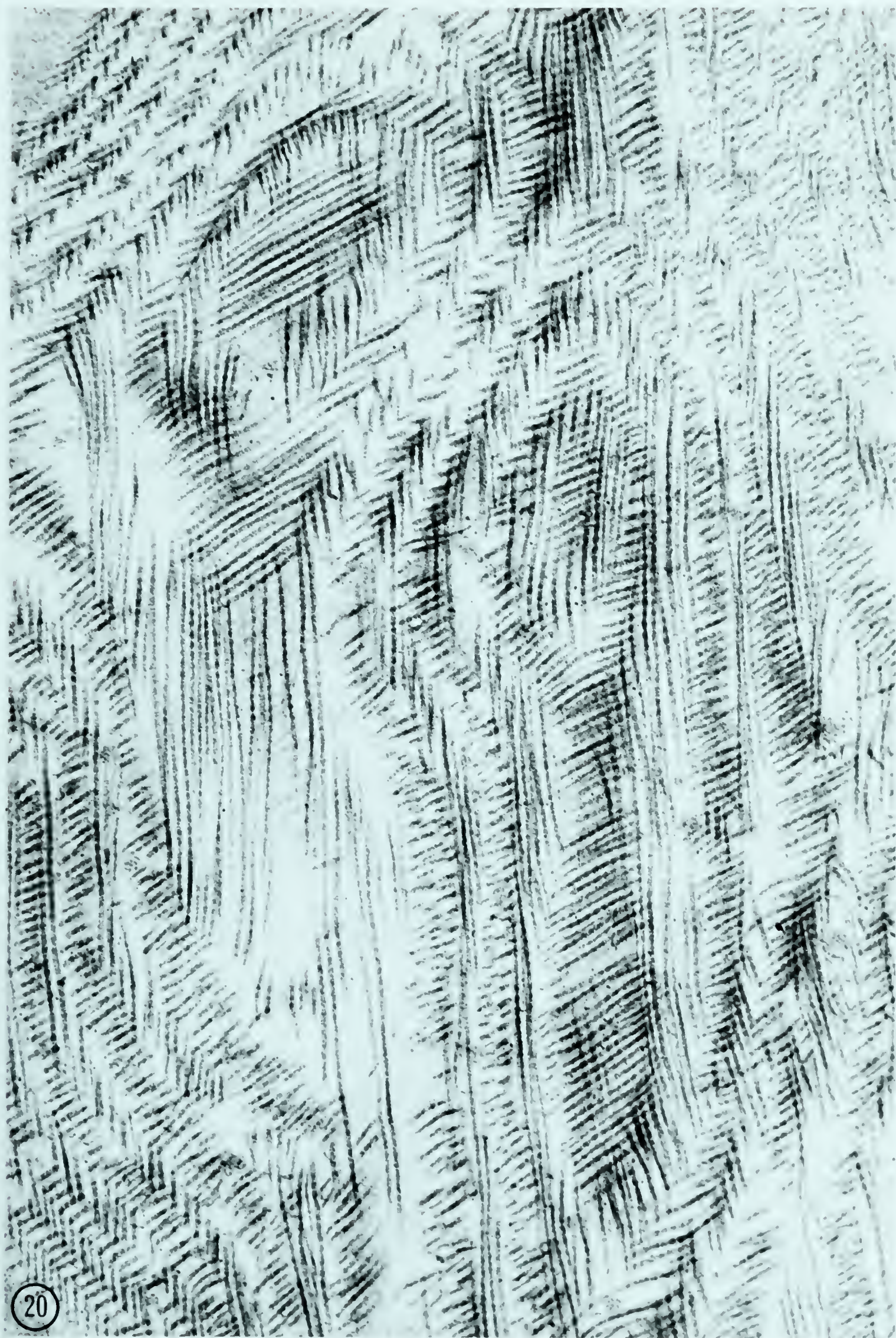


Fig. 21. A vertical section showing the basal region of the basal cell (BC) and a part of the basement lamella. In addition to regular horizontal arrangement of the collagen fibrils, sometimes some collagen fibrils extend in the vertical direction. On the underside of the basement lamella (BL) are large vacuoles (VA) present in the fibroblasts.

Glutaraldehyde-osmium tetroxide fixation, Araldite embedding, uranyl acetate staining. X 37,000.

Fig. 22. A vertical section through the basal region of a basal cell (BC) and the basement lamella (BL). Within the basement lamella sometimes are seen processes (FP) of fibroblasts (FB). The fibroblasts show large vacuoles (VA). Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 19,800.

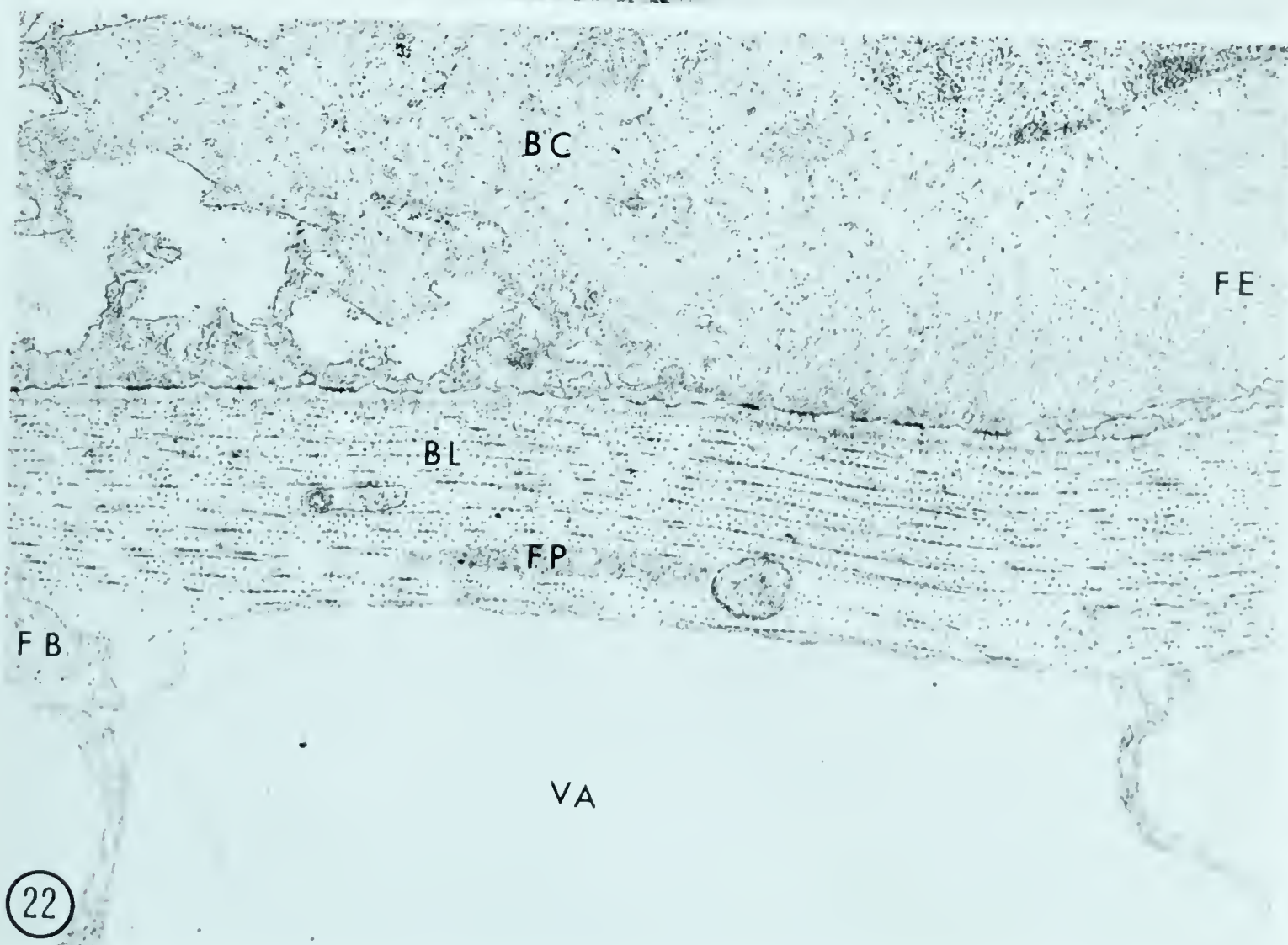
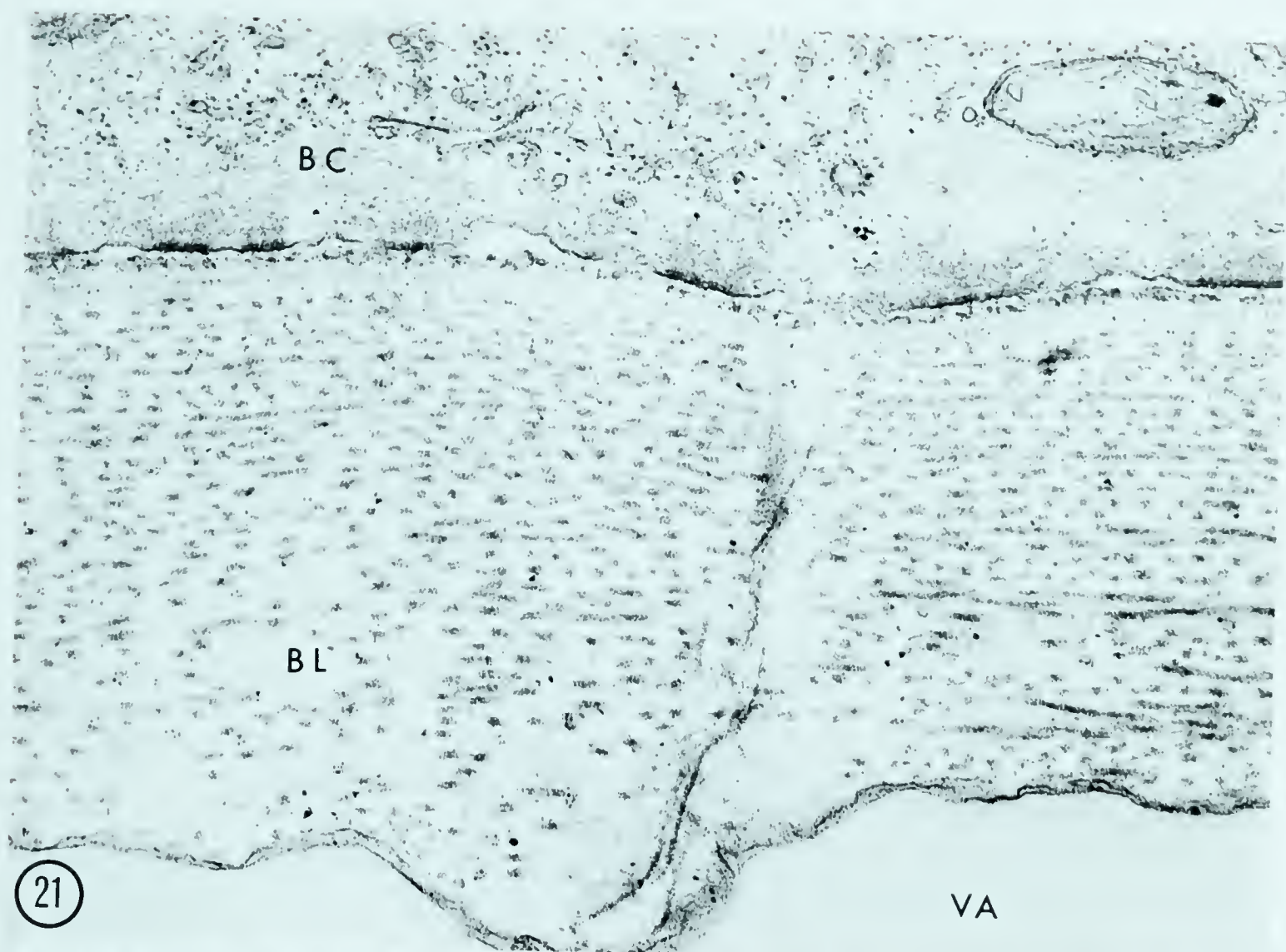


Fig. 23. A vertical section through the basal region of the basal cell (BC) and the basement lamella (BL). This material was stained overnight in 10 percent PTA at PH 5.4 immediately after fixation. Note the cavities in the adepidermal space (AS) and an indistinct dermal membrane (DM).

Osmium tetroxide fixation, methacrylate embedding. X 55,000.

Fig. 24. A vertical section through the dermo-epidermal junction and the surrounding area. Note the adepidermal space (AS) occupied by flake-shaped structures. The basement lamella (BL) does not show collagen fibrils because this section is unstained. Below the basement lamella is part of a fibroblast (FB). The dermal membrane is also not visible.

Osmium tetroxide fixation, Araldite embedding and unstained. X 75,000.

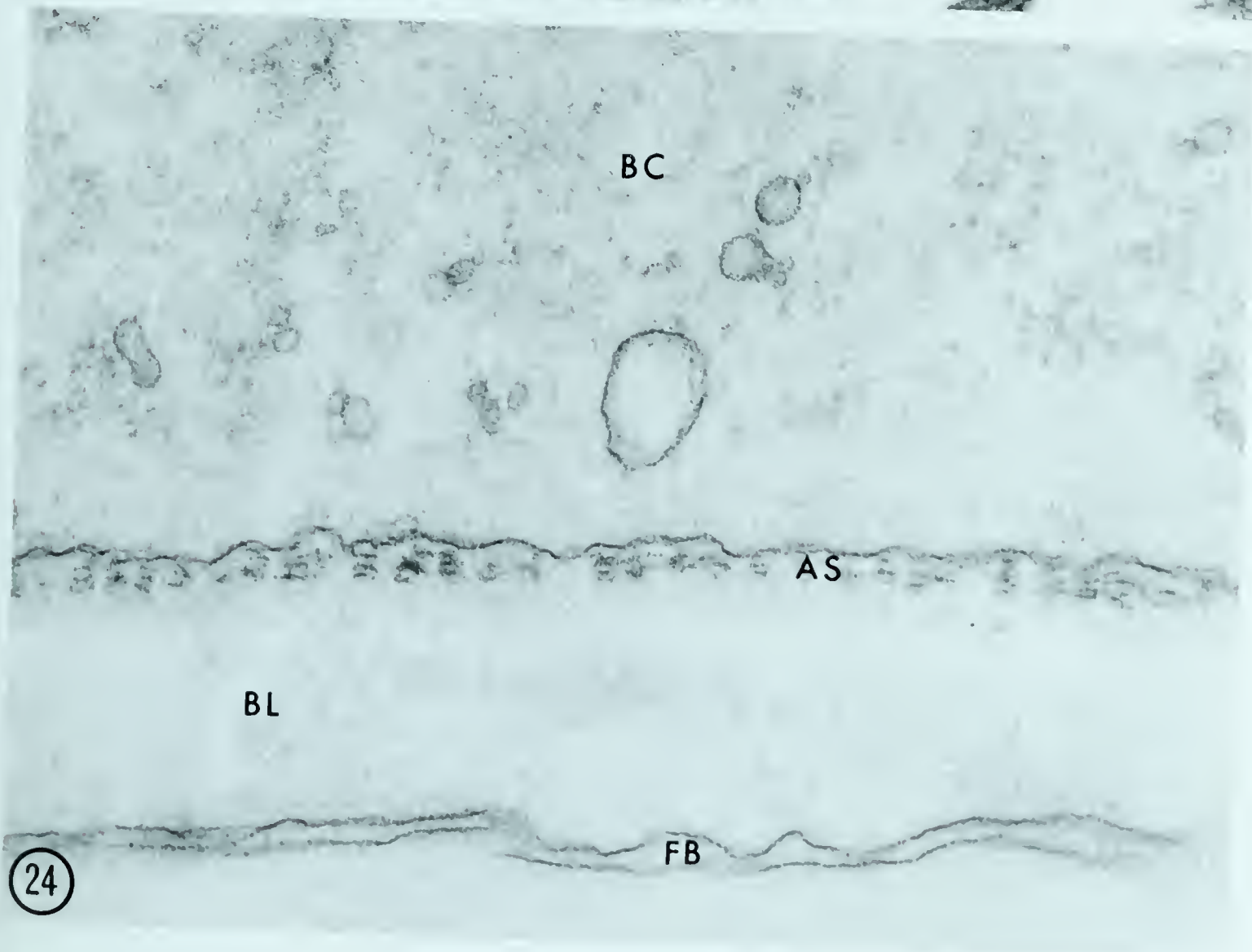
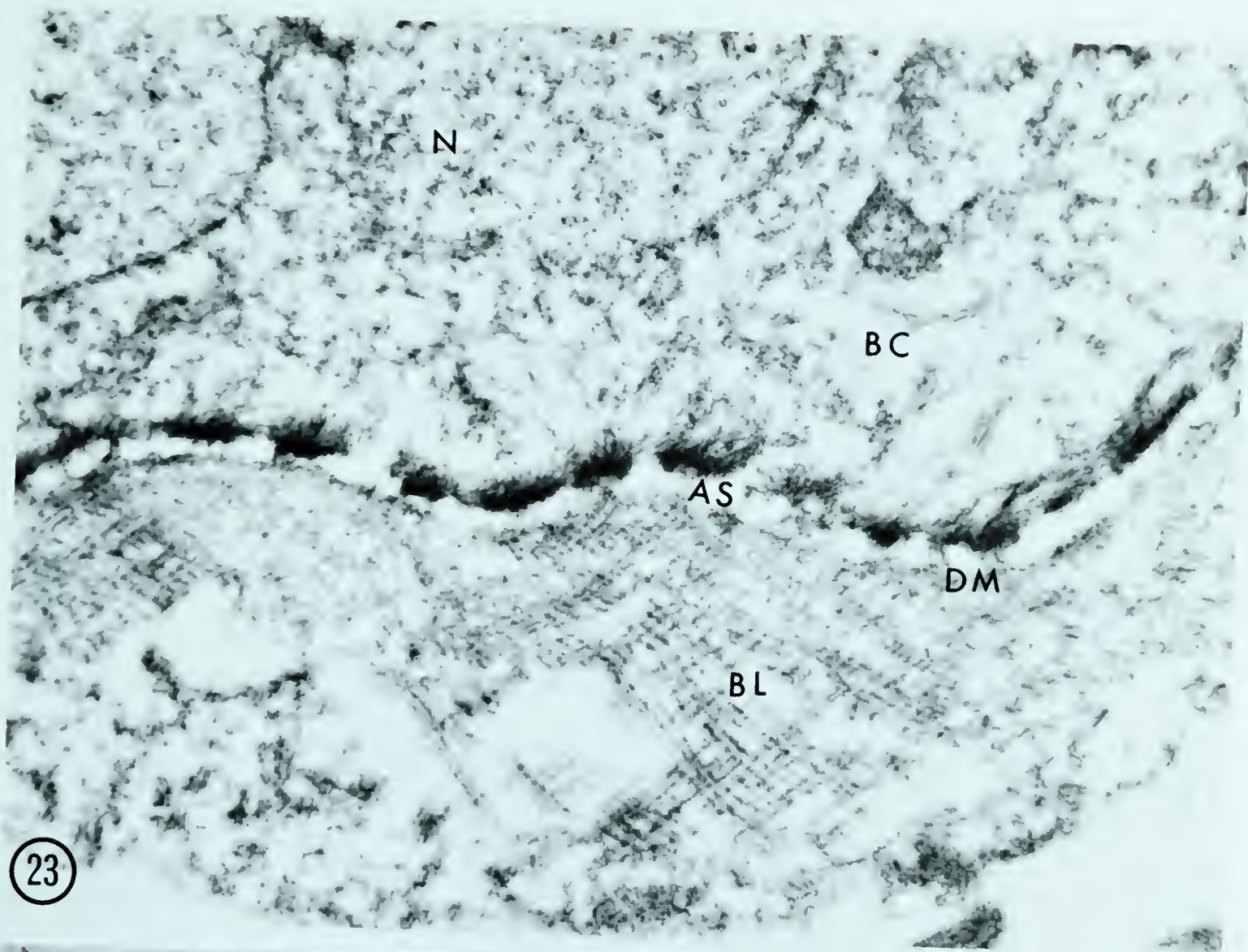


Fig. 25. A vertical section through the basal region of the basal cell, dermo-epidermal junction and a part of the basement lamella (BL). The basal cell membrane (BCM) is separated from the dermal membrane (DM) by adepidermal space (AS). The adepidermal space is occupied by a patchy electron opaque material. Glutaraldehyde-osmium tetroxide fixation, Araldite embedding and lead hydroxide staining. X 80,000.

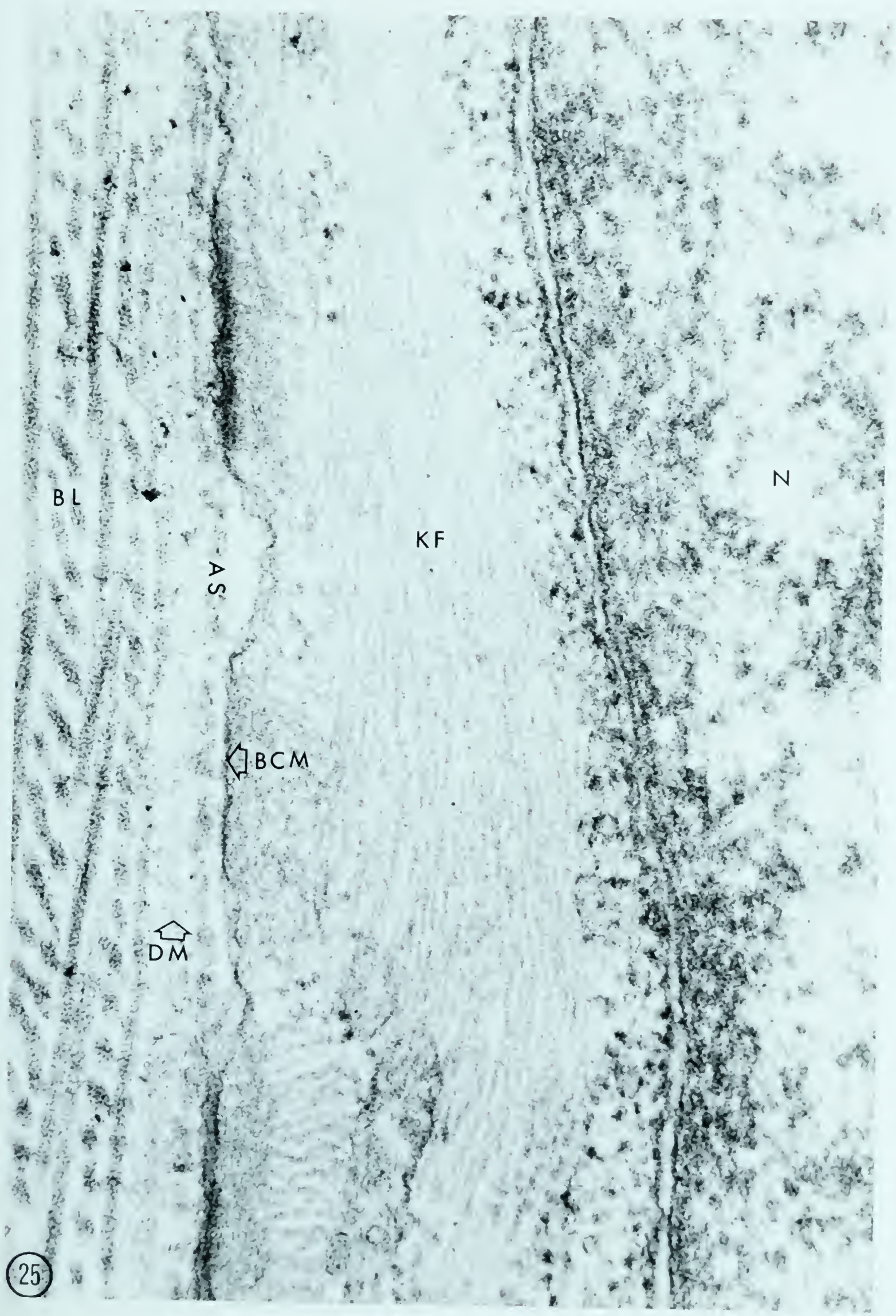


Fig. 26. A vertical section through the skin treated for one hour with hyaluronidase. Note the desmosomes (DS) splitting apart and no increase in the intercellular spaces (IS). Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 21,500.

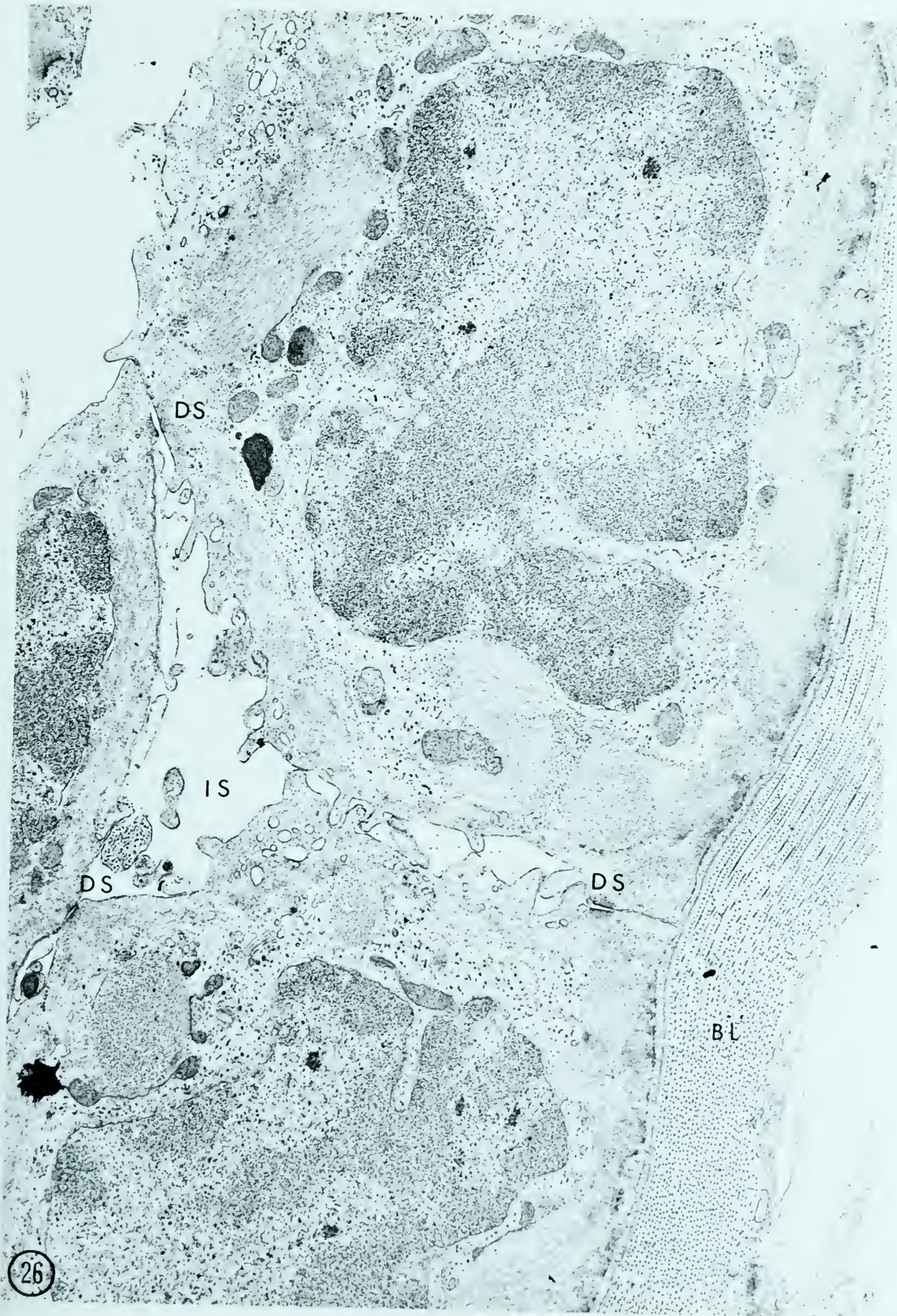


Fig. 27. Same as figure 26. Note the desmosome (DS) has split apart while the contiguous plasma membranes at other points are still close together.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 21,500.

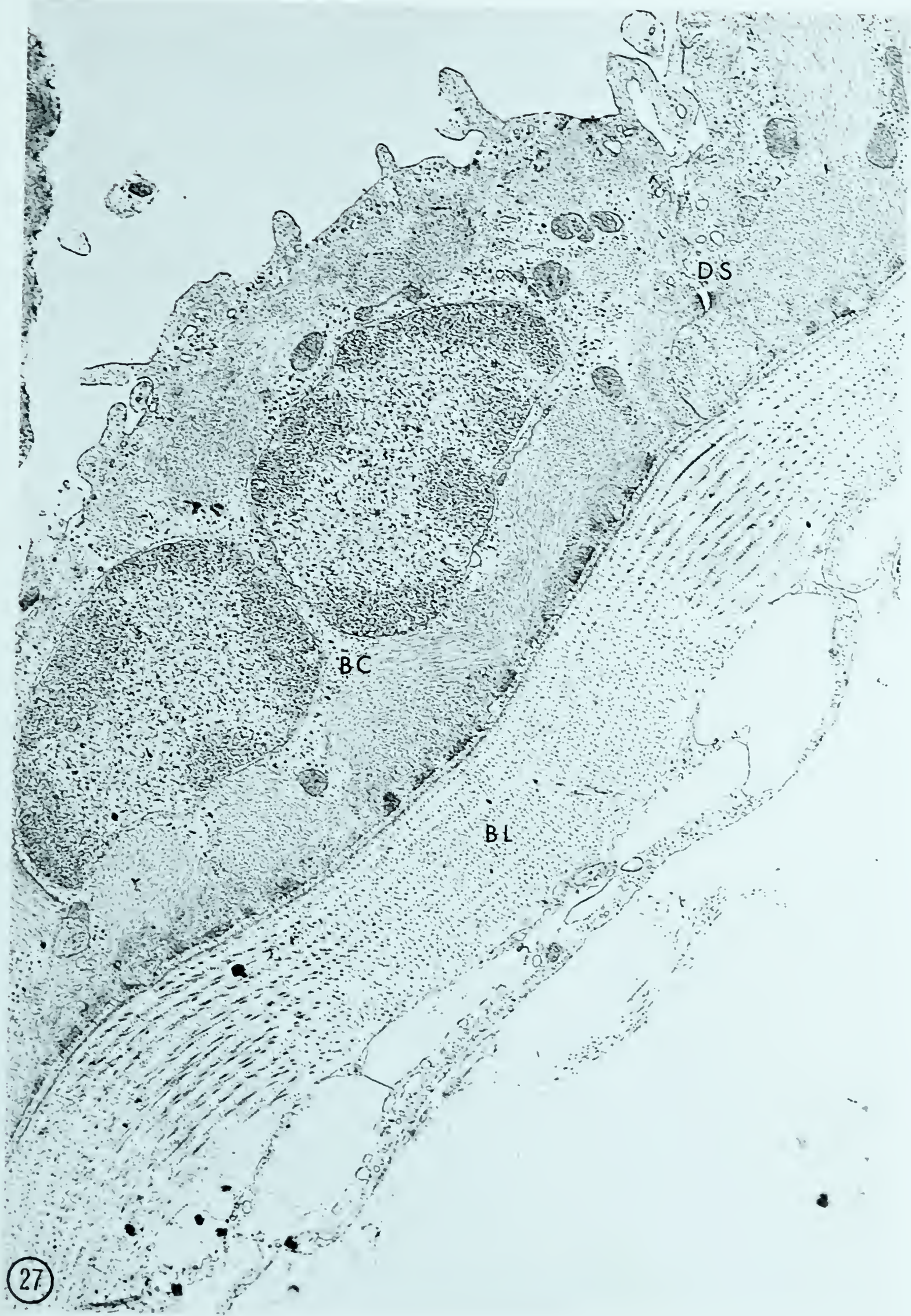


Fig. 28. Same as figure 26. Note that in addition to the desmosomes coming apart, the adepidermal space (AS) is also collapsing and the basal cell membrane shows bulgings towards the dermal membrane (DM) .
Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 21,500.

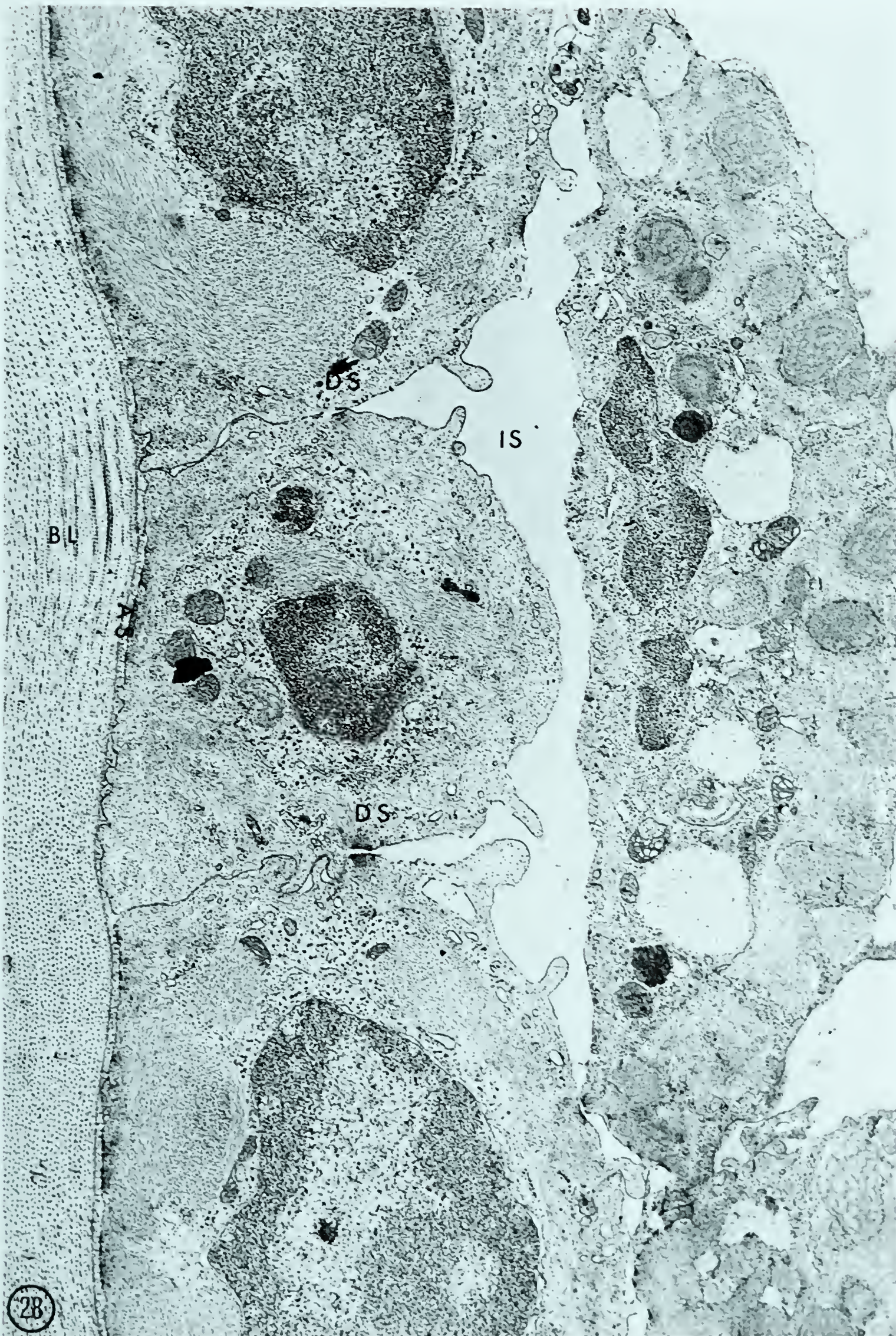


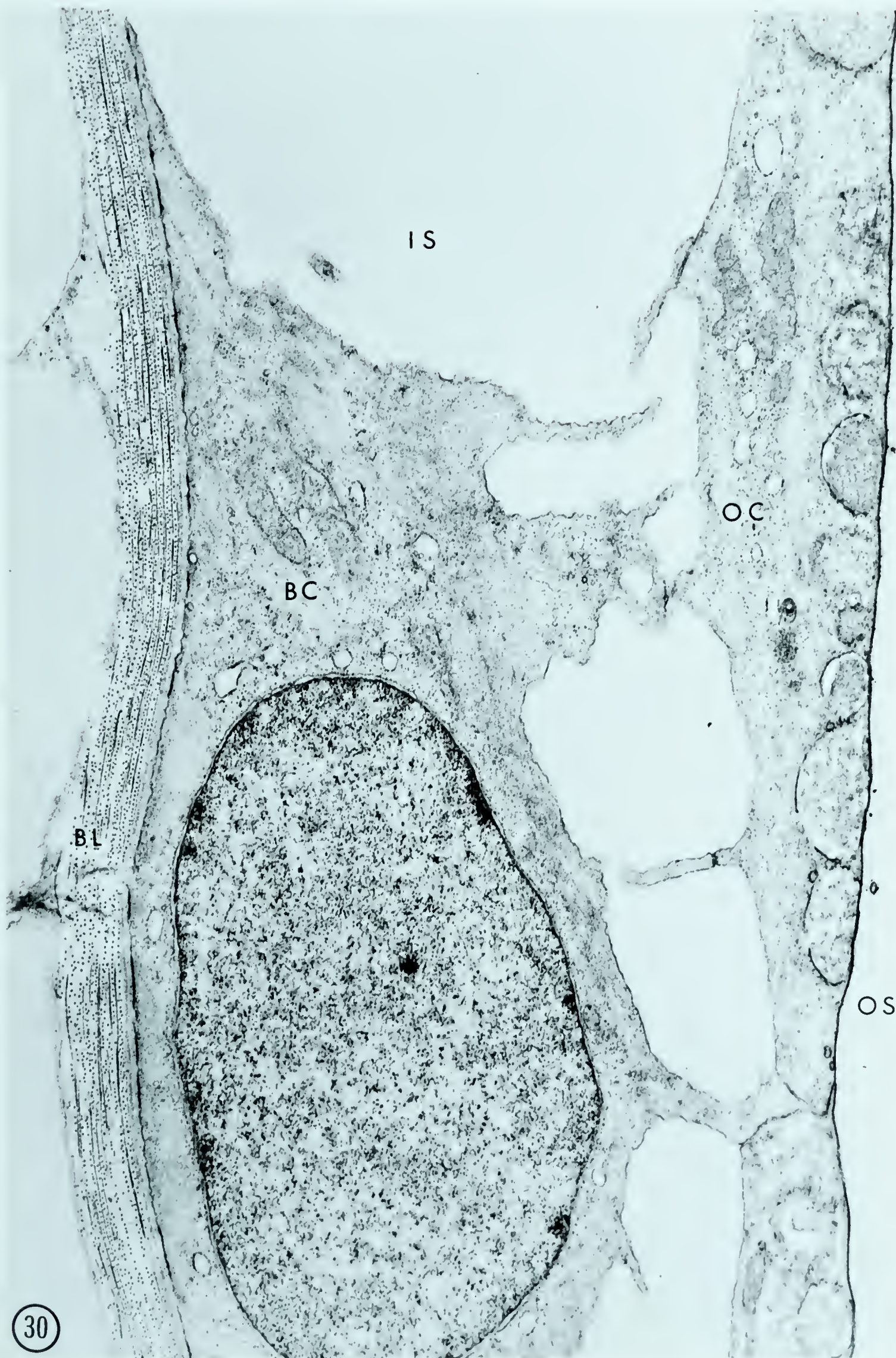
Fig. 29. A light micrograph of a transverse section through the tail of tadpole fixed after treatment with chlorinated tapwater for ten minutes. Only dorsal part is shown. Note the blisters formed between the epidermis and basement lamella. Zenker's fixation and Iron haematoxylin staining. X 580.



Figs. 30-32. Vertical sections of skin fixed after treatment with chlorinated tapwater for ten minutes. Note the increase in the intercellular spaces and the pulled intercellular bridges. The cisternae of endoplasmic reticulum may also be seen to be swollen.

BC: basal cell; BL: basement lamella;
IS: intercellular space; OC: outer cell;
OS: outside.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 14,000.



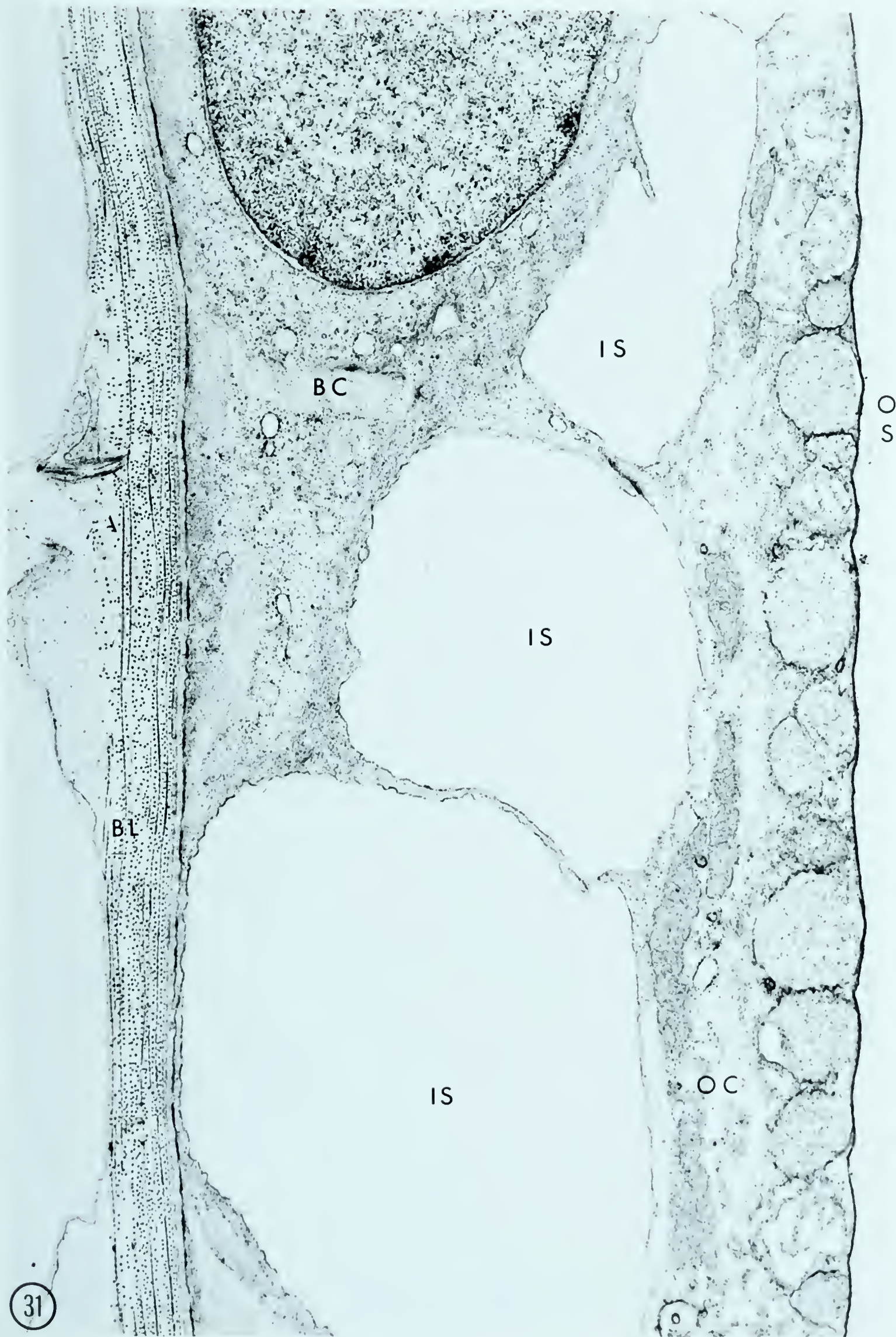




Fig. 33. A vertical section through the skin fixed after treatment with chlorinated tapwater for fifteen minutes. Note the ruptured basal cell with its swollen mitochondria (M). The plasma membrane of the basal cell (BCM) is still intact with the basement lamella. The collagen fibrils in the basement lamella are indistinct because the section is unstained.

Osmium tetroxide fixation, Araldite embedding and unstained. X 21,000.

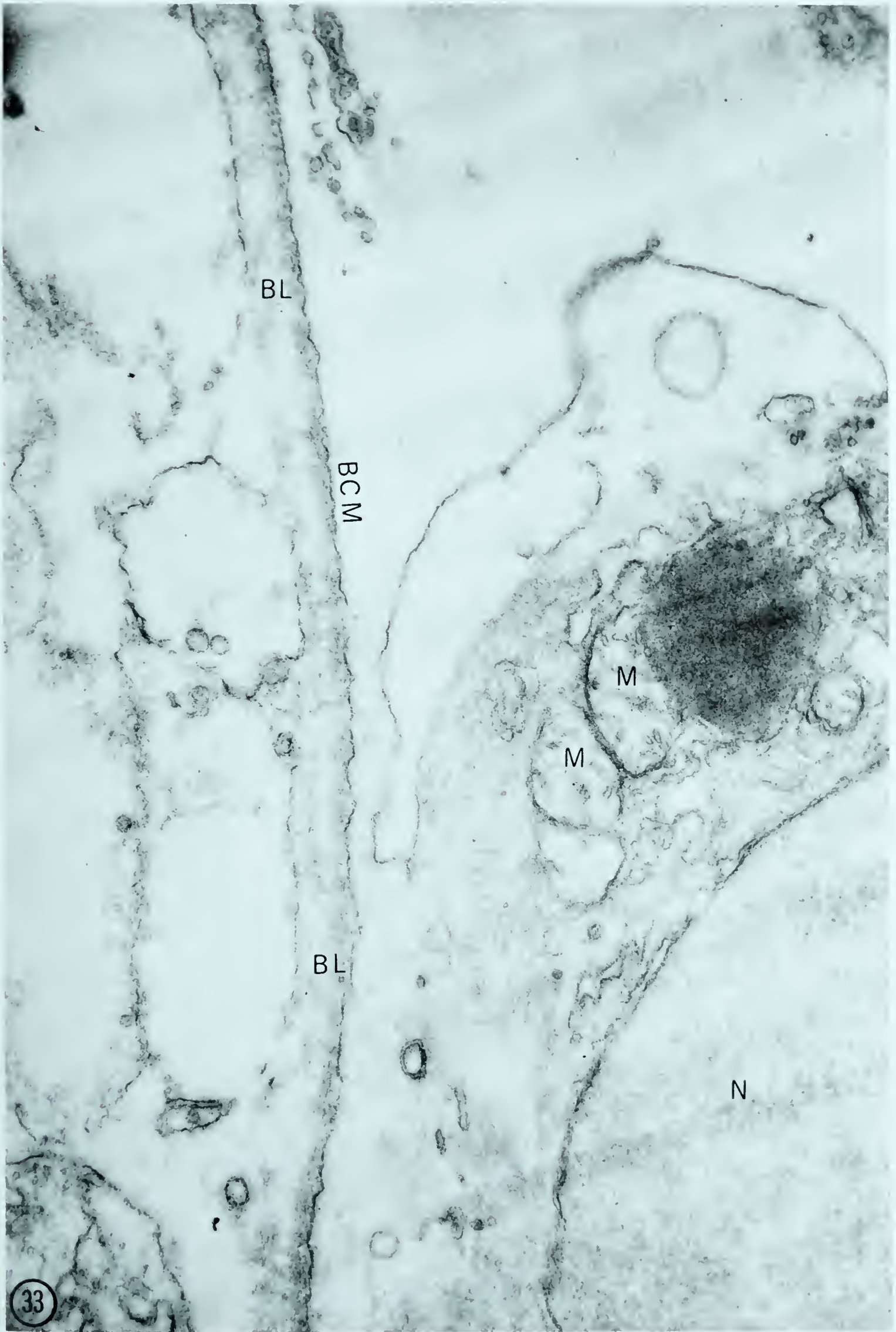
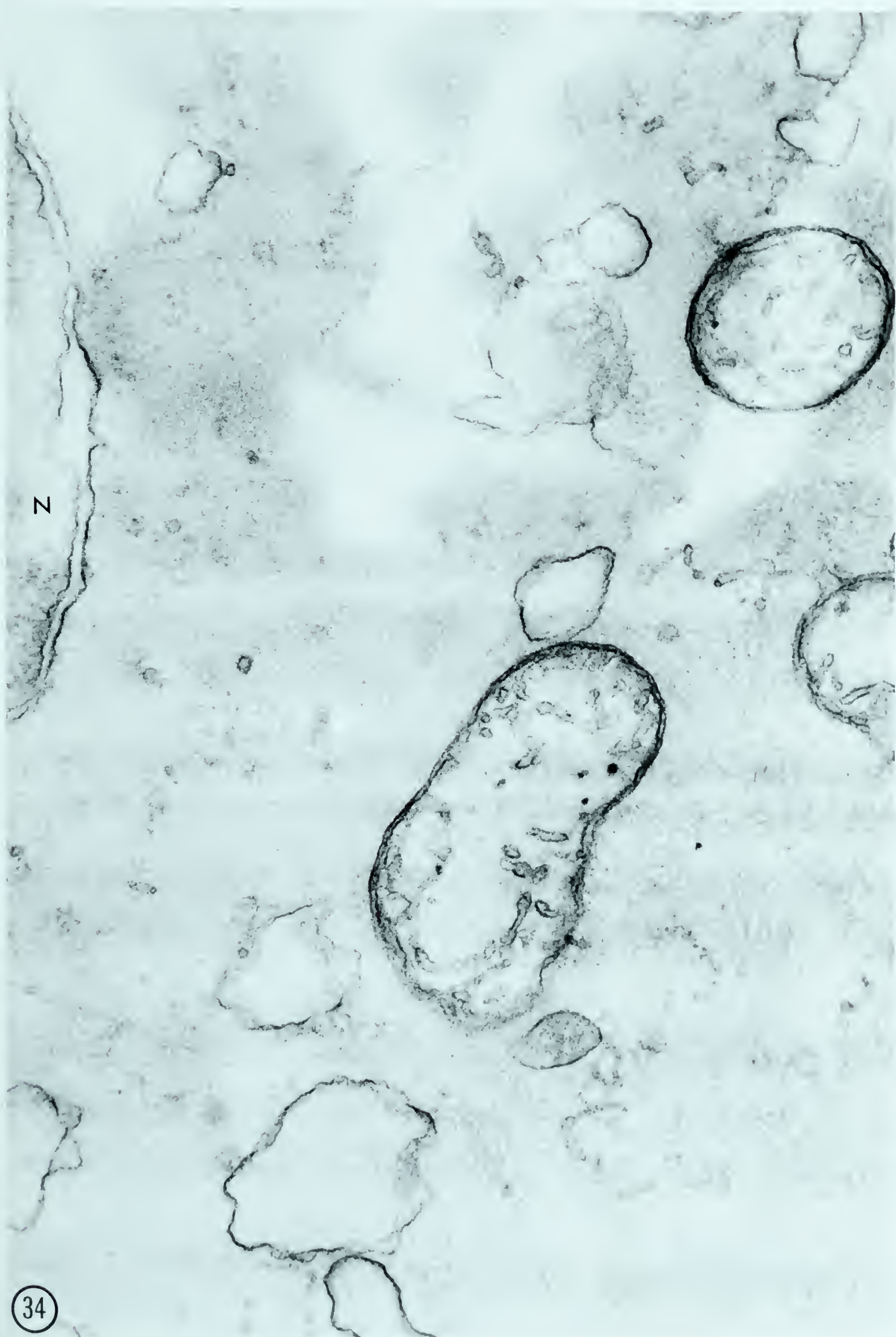


Fig. 34. A section through an outer cell showing swollen mitochondria (M) and space developed in the double nuclear envelope. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 21,000.



Z

Fig. 35. A section through an outer cell showing a general loss of regular arrangement in the keratin filaments (KF), and swollen mitochondria (M).
Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 22,000.

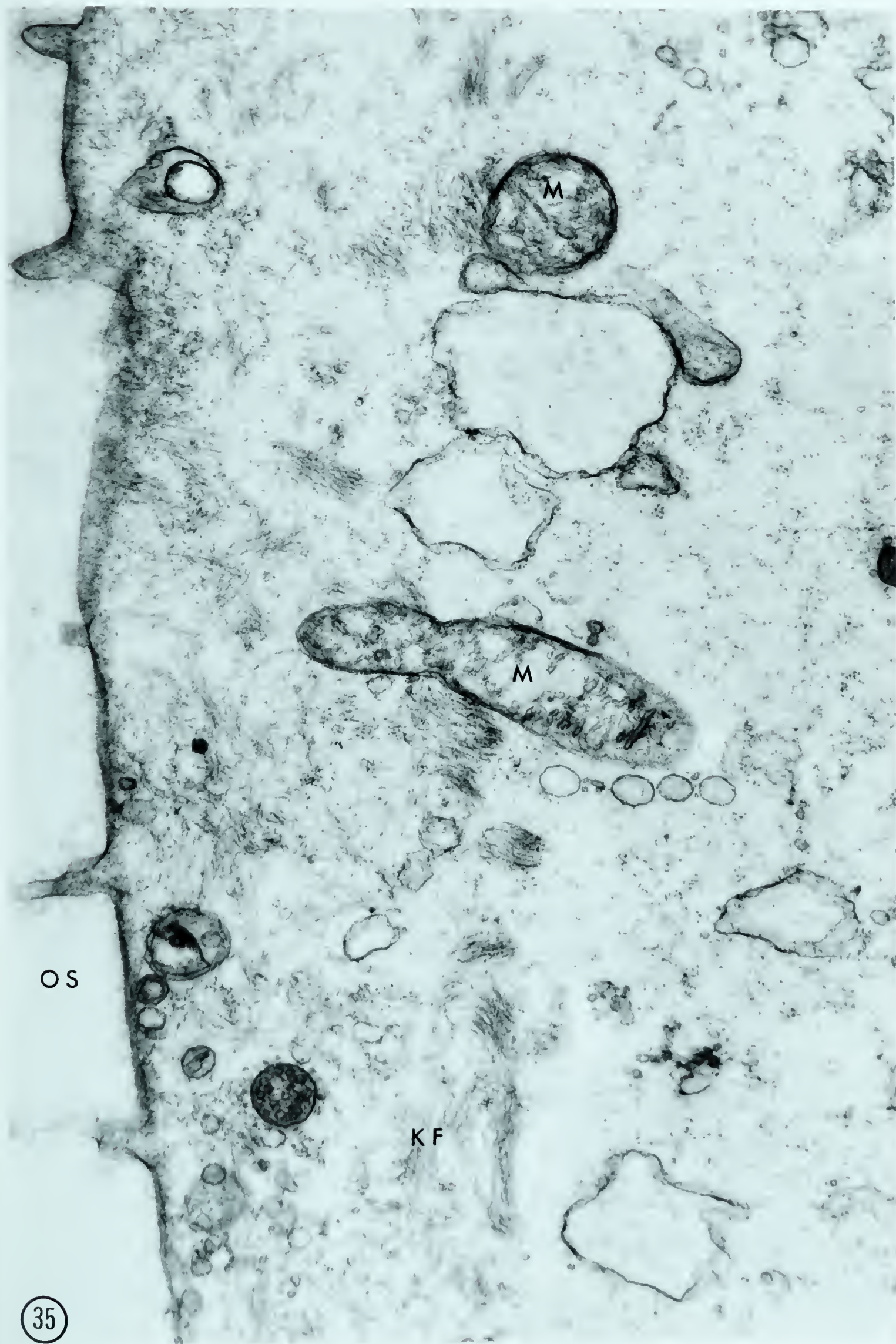


Fig. 36. A diagrammatic figure of an anuran tadpole after Wright (1951) to show the arrangement of lateral-line organs. X 9.

A: Angular; D: dorsal; IO: infra-orbital;
M: mid-lateral; ML: mandibular; SO: supra-orbital; V: ventral.

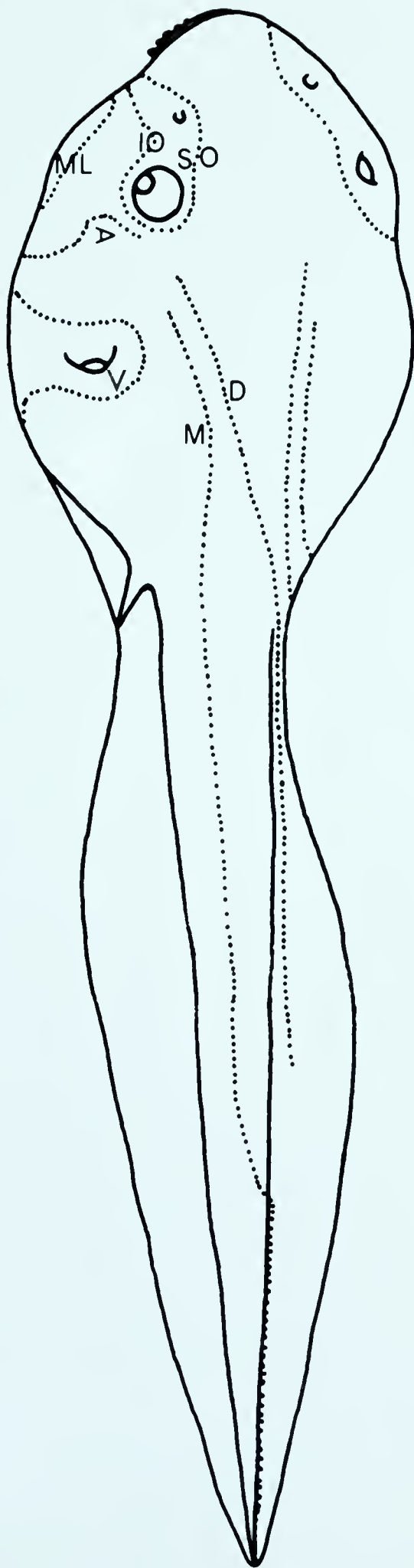


Fig. 37. A montage of many electron micrographs.

It shows a vertical section passing through the center of the lateral-line organ.

BC: basal cell; BL: basement lamella; EC: epidermal cell; G: Golgi complex; MC: mantle cell; NE: nerve ending; RC: receptor cell; SC: sustentacular cell.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate stained. X 10,000.

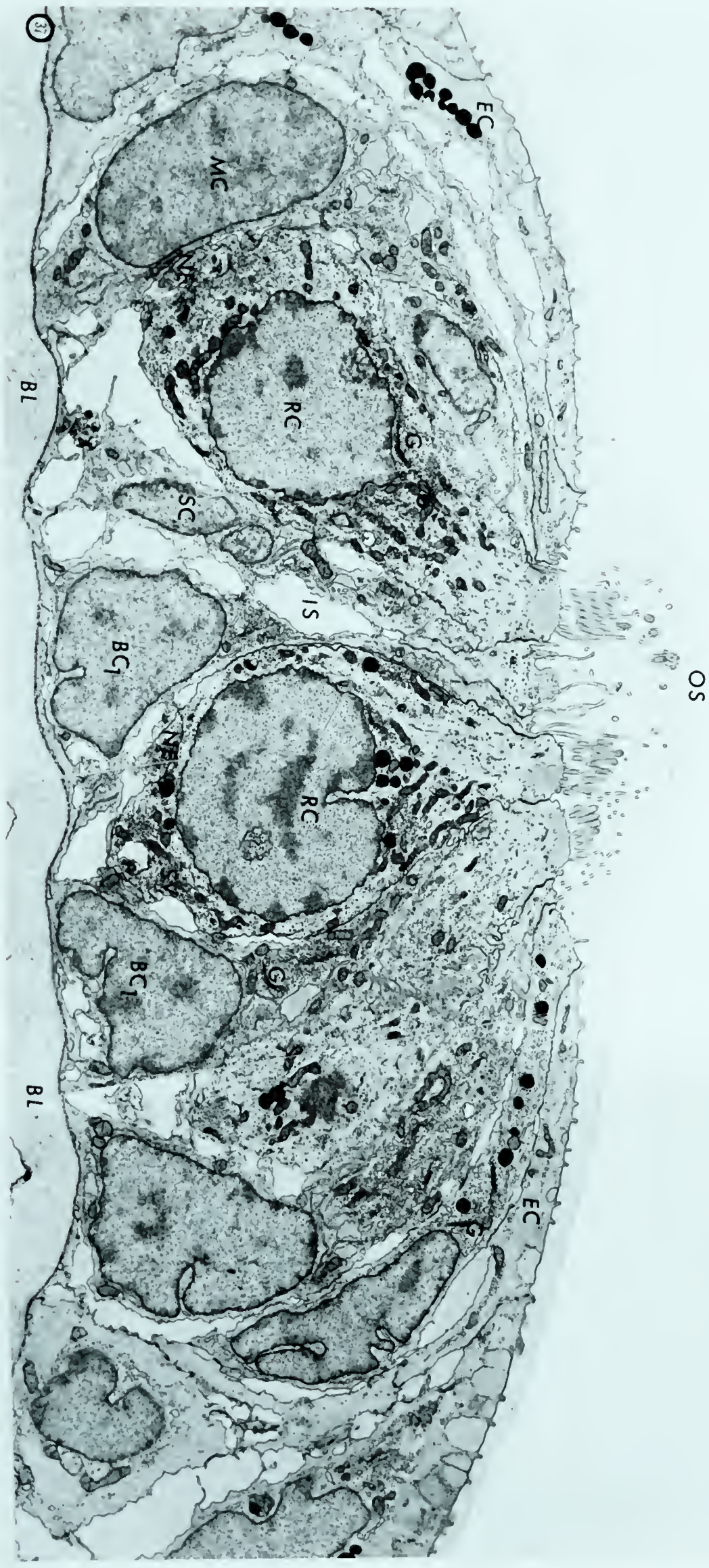


Fig. 38. A vertical section passing through the apical region of the receptor cell.
CP: cuticular plate; K: kinocilium;
ML: microvillus; S: stereocilium.
Osmium tetroxide fixation, Araldite
embedding, uranyl acetate staining.
X 21,000.

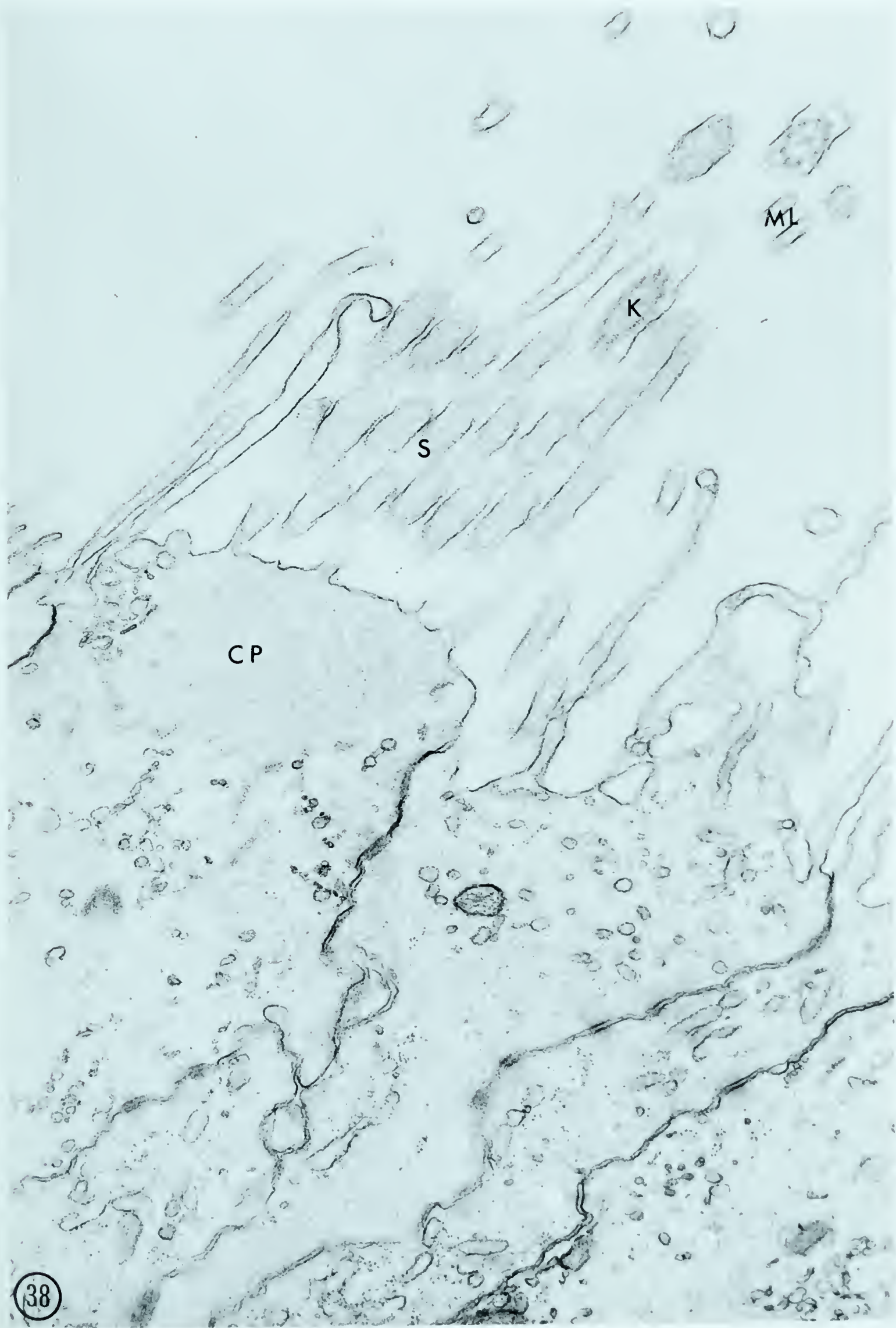


Fig. 39. Same as figure 38 but using a different fixation.

CP: cuticular plate; ML: microvillus;

MT: microtubules; S: stereocilium.

Glutaraldehyde-osmium tetroxide fixation,

Araldite embedding, uranyl acetate and

lead hydroxide staining. X 34,500.

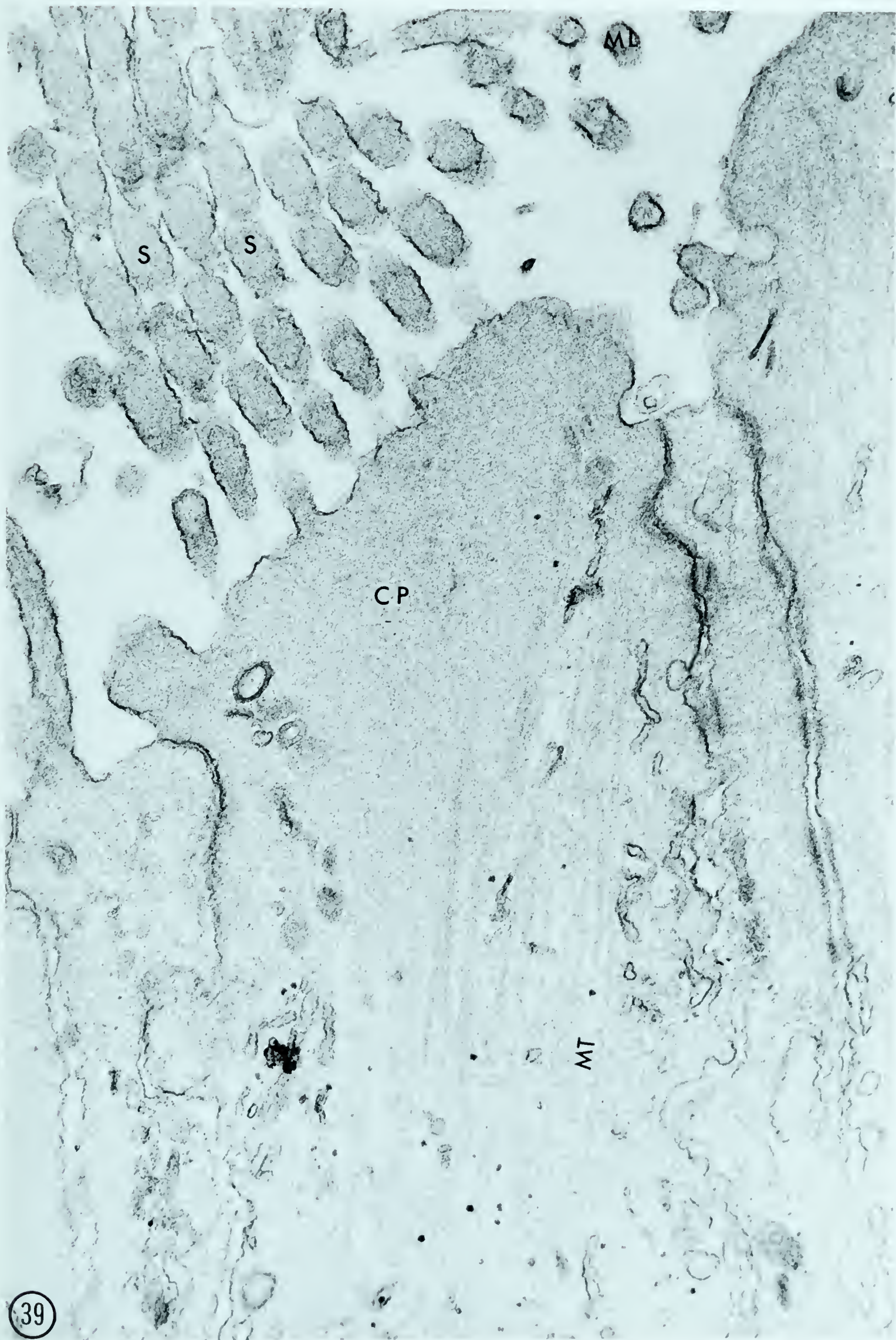


Fig. 40. A section parallel to the surface of the skin cutting the various outgrowths transversely. Note six kinocilia (K) each with its characteristic structure, and stereocilia (S) and microvilli (ML). The various outgrowths are arranged in groups. Each group arises from one cell and consists of one kinocilium (K) few stereocilia and some microvilli (ML). The kinocilium is always situated towards the center of the organ.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 15,700.

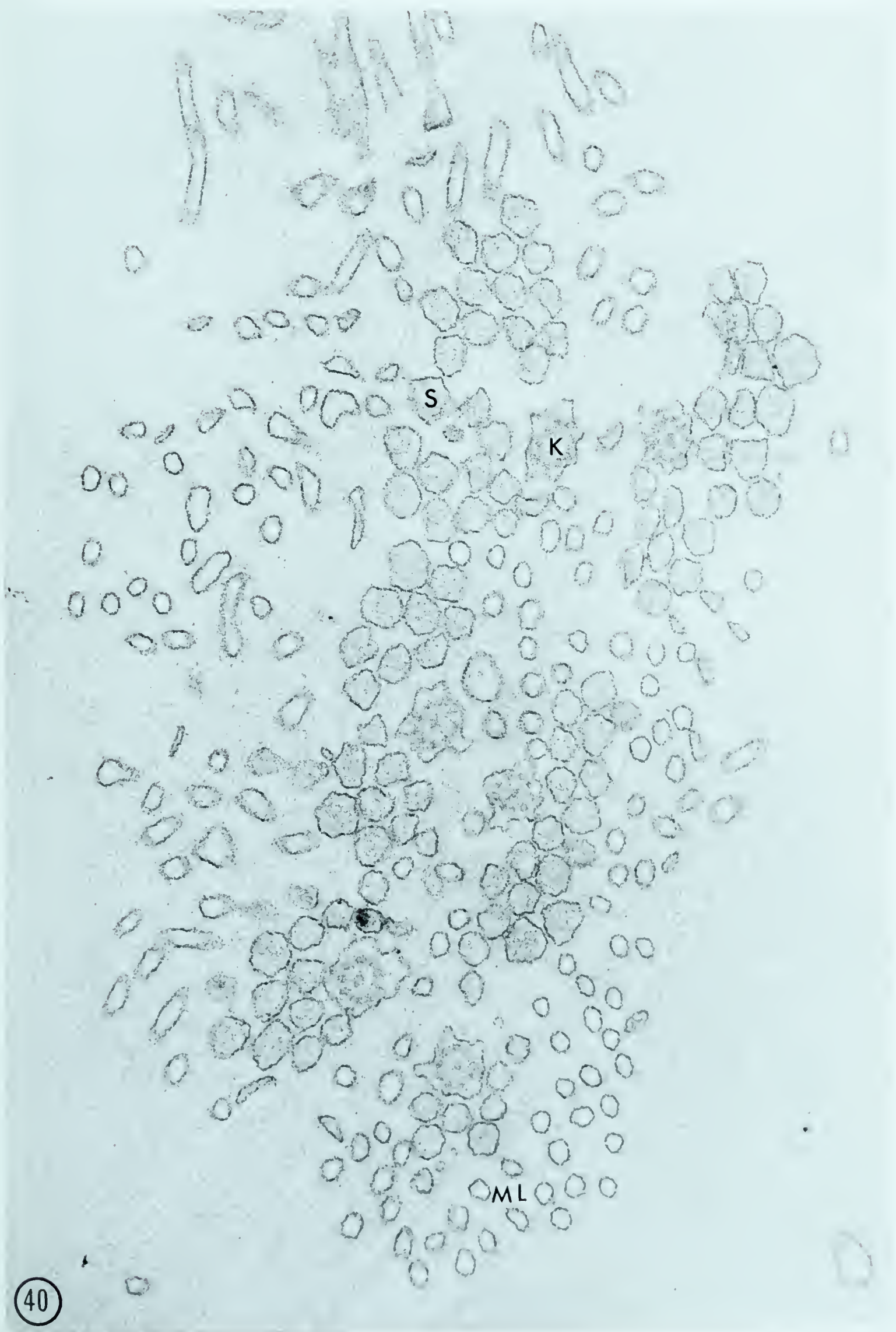


Fig. 41. A section through the kinocilia and stereocilia. Tangential sections through the stereocilia show striations but in a longitudinal section filaments can be made out.

Glutaraldehyde-osmium tetroxide fixation, Araldite embedding and uranyl acetate and lead hydroxide staining. X 36,500.

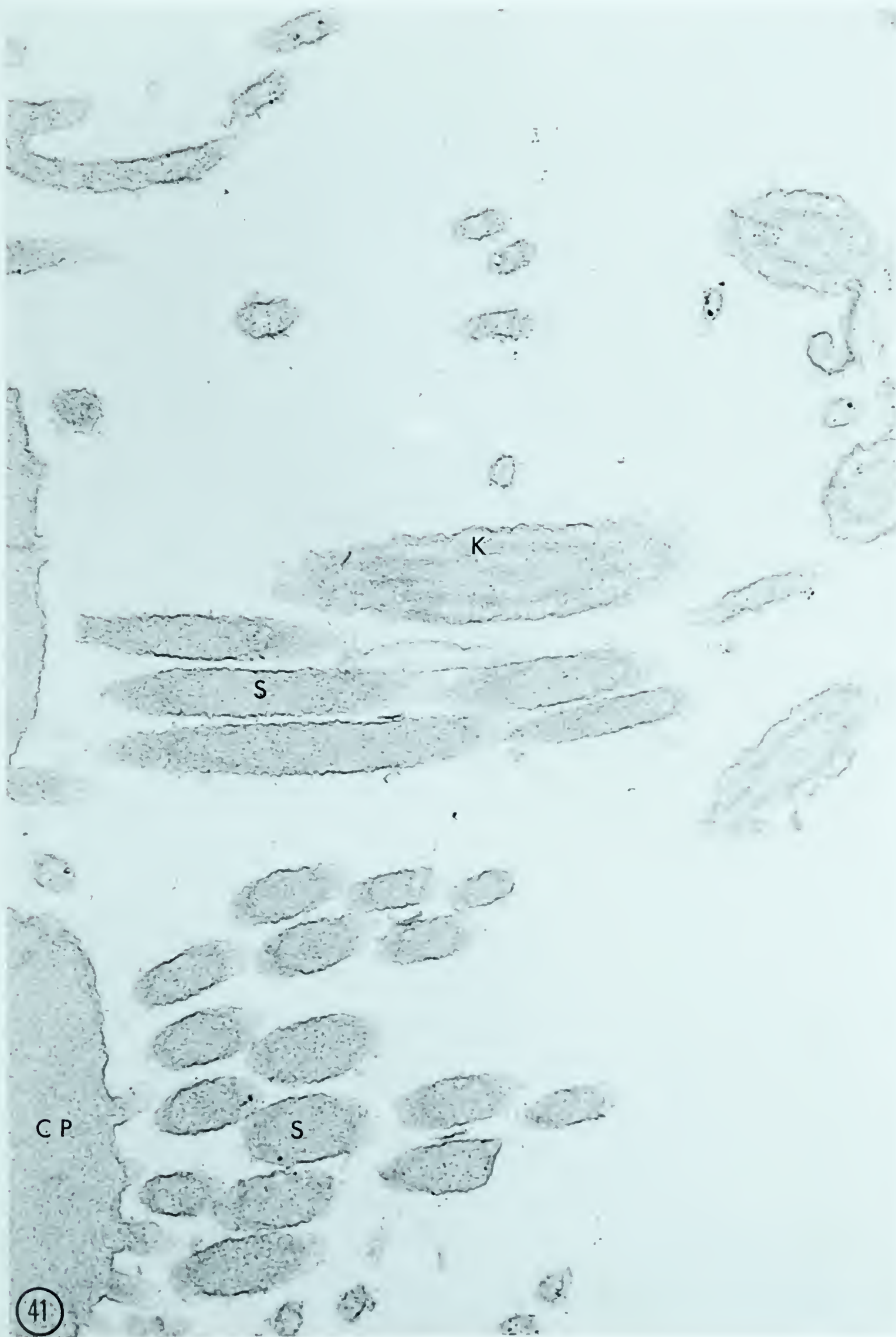


Fig. 42. A vertical section through the apical portions of receptor cells. The cuticular plates (CP) show a network of filaments about 50 Å^o in diameter which resemble the filaments in the stereocilia. From the underside the cuticular plates microtubules (MT) take their origin. Glutaraldehyde-osmium tetroxide fixation, Araldite embedding, uranyl acetate and lead hydroxide staining. X 37,000.

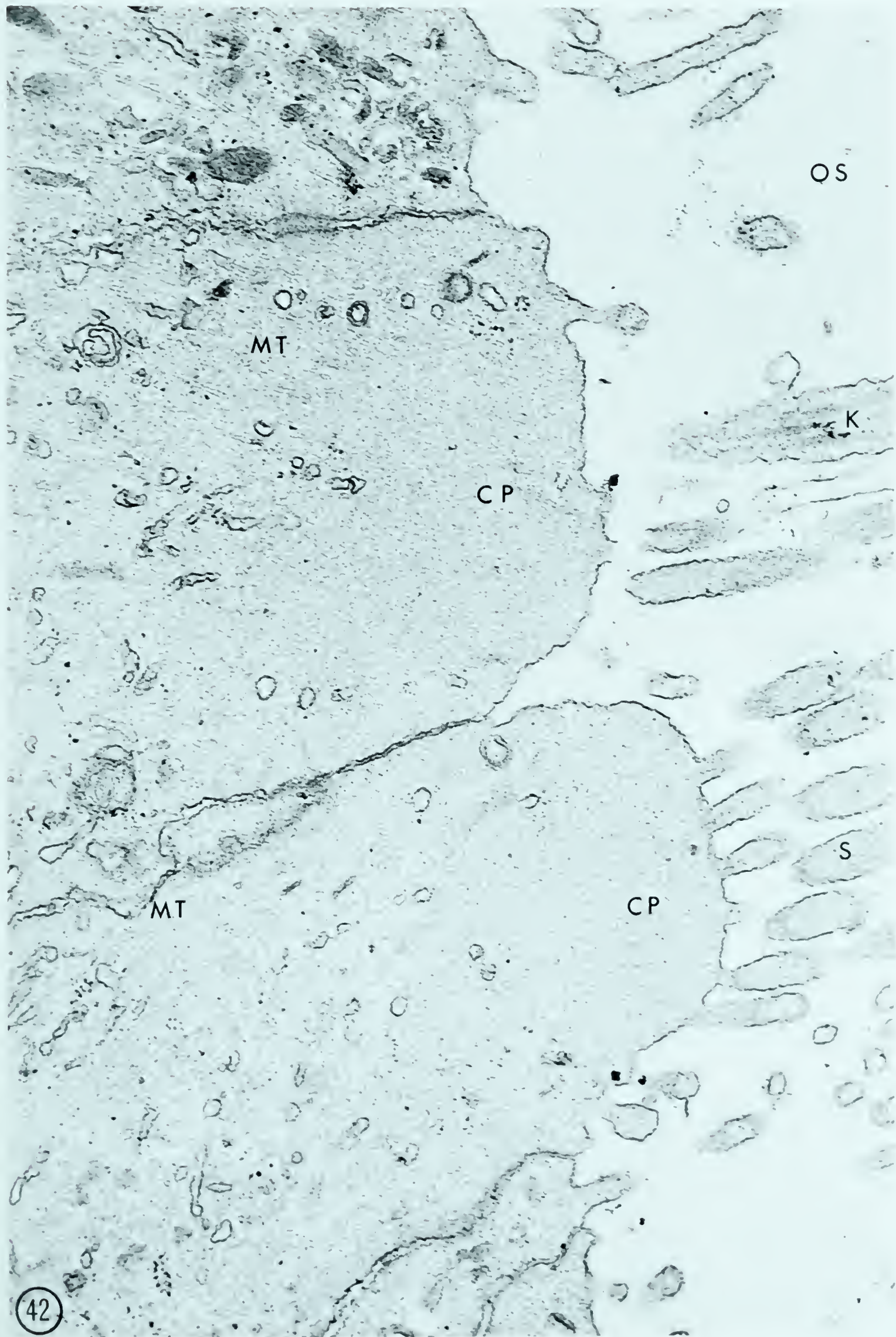


Fig. 43. A vertical section through the medial region of the receptor cell showing the microtubules (MT) which take their origin from under the cuticular plate and penetrate deep into the cell in bundles. It also shows a Golgi complex (G), synaptic vesicles (SV), mitochondria (arranged somewhat parallel to the microtubules) and endoplasmic reticulum (ER). Glutaraldehyde-osmium tetroxide fixation, Araldite embedding, uranyl acetate and lead hydroxide staining. X 70,000.

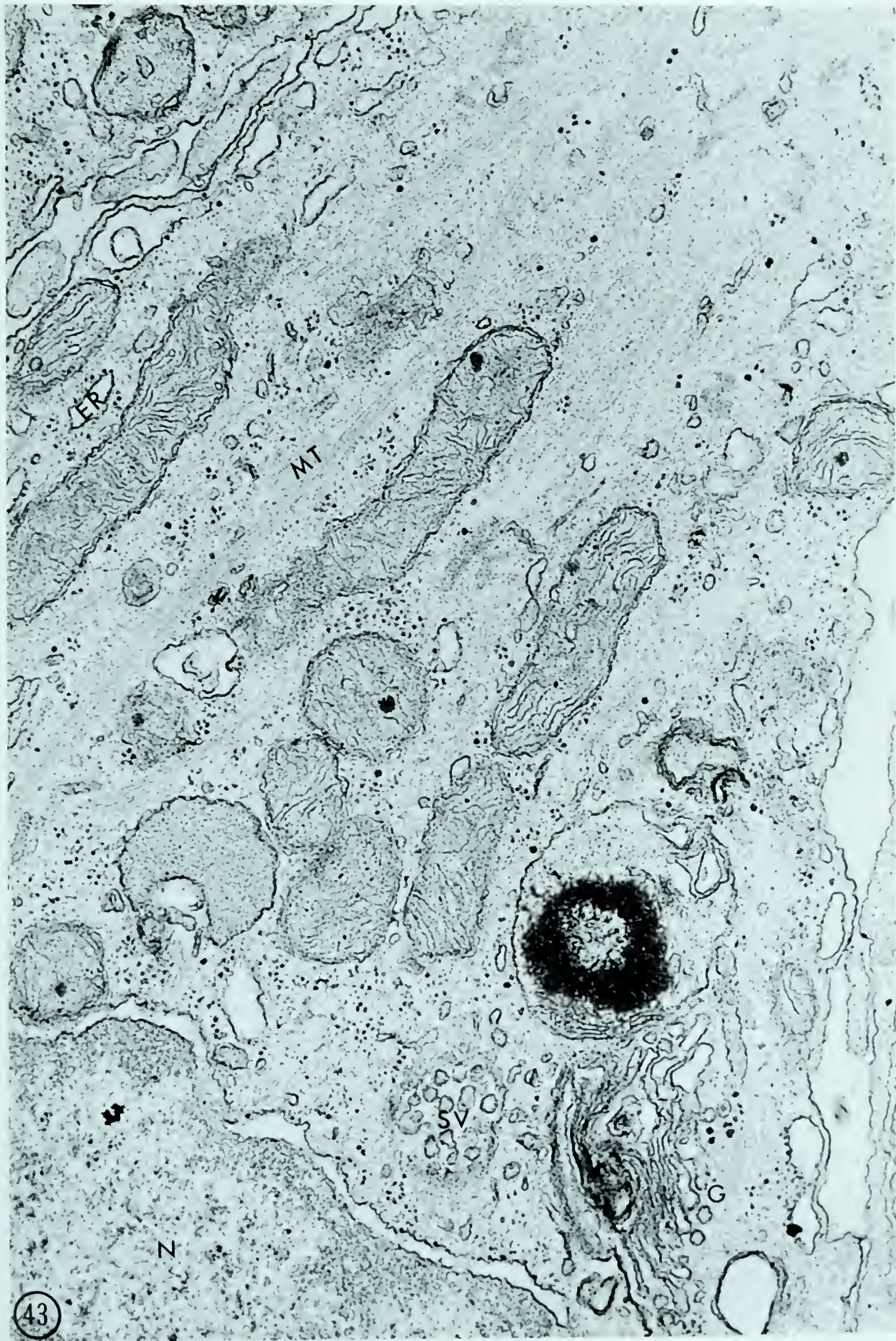


Fig. 44. A vertical section through the basal half of the receptor cell showing the microtubules (MT) synaptic vesicle (SV) and nerve endings (NE).

IS: intercellular space.

Glutaraldehyde-osmium tetroxide fixation,
Araldite embedding, uranyl acetate and lead
hydroxide staining. X 21,000.

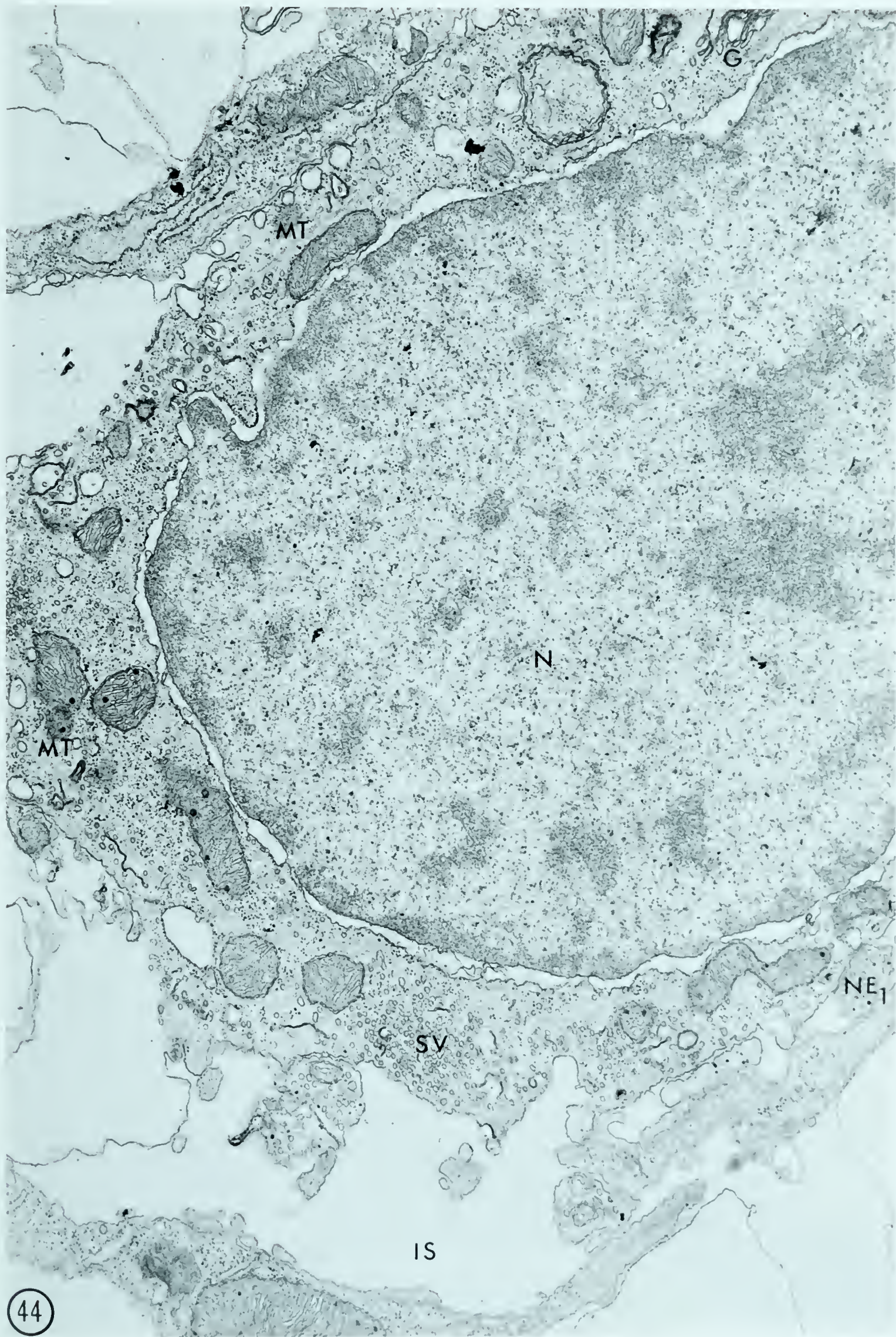


Fig. 45. A part of the basal region of the receptor cell showing synaptic vesicles (SV), microtubules (MT) and the nerve ending (NE) surrounded by the intercellular space (IS) which abounds in this region. Glutaraldehyde-osmium tetroxide fixation, Araldite embedding, uranyl acetate and lead hydroxide staining. X 37,500.

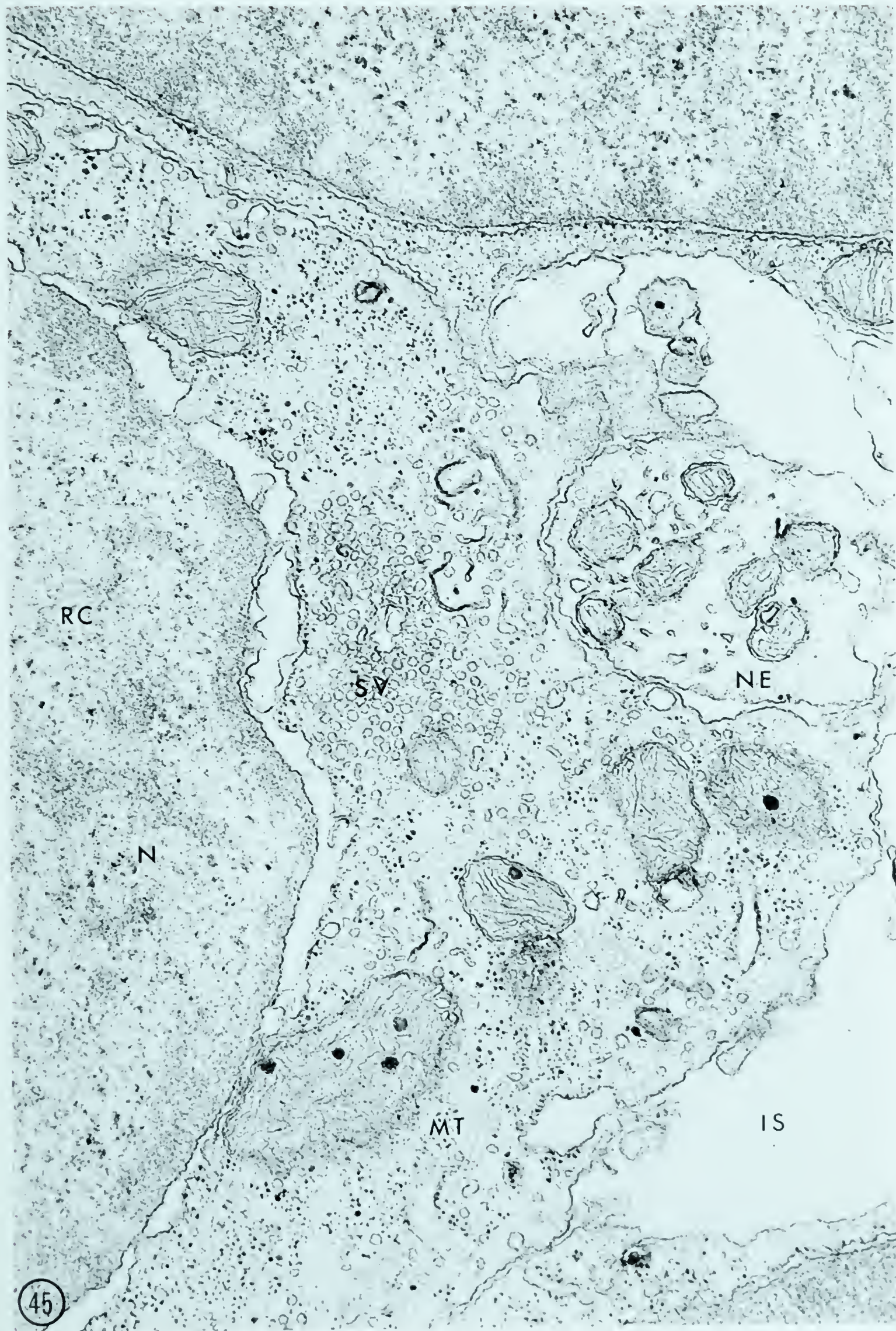


Fig. 46. A vertical section through the basal region of the lateral-line organ showing a part of a receptor cell (RC), a basal cell (BC). The receptor cell is in association with two types of nerve endings (NE₁ and NE₂). BL: basement lamella; IS: intercellular space; SV: synaptic vesicles. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 22,400.

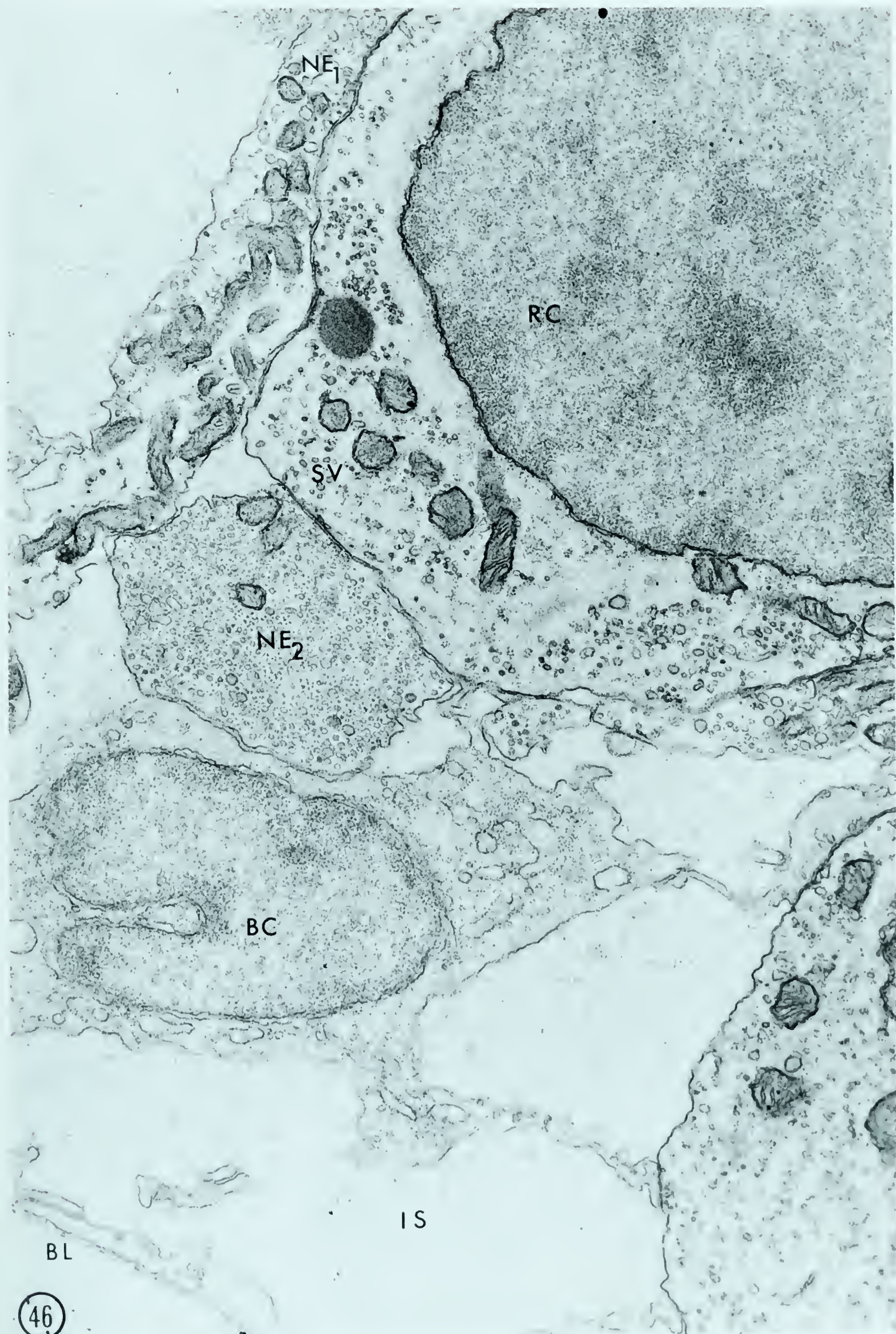


Fig. 47. A section showing a portion of the receptor cell (RC) and a portion of nerve ending (NE₂). In the receptor cell below the receptor cell membrane is present a system of membranes and sacs (SA).

IS: intercellular space; M: mitochondrion;
N: nucleus of receptor cell; SCL: synaptic cleft; SV: synaptic vesicles.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 75,000.



Fig. 48. A section in the plane of the skin through the lateral-line organs showing the inter-relationship of various types of cells and the nerve endings.

MC: mantle cell; NE₁: afferent nerve ending;
NE₂: efferent nerve ending; RC: receptor cell;
SC: sustentacular cell.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 12,400.

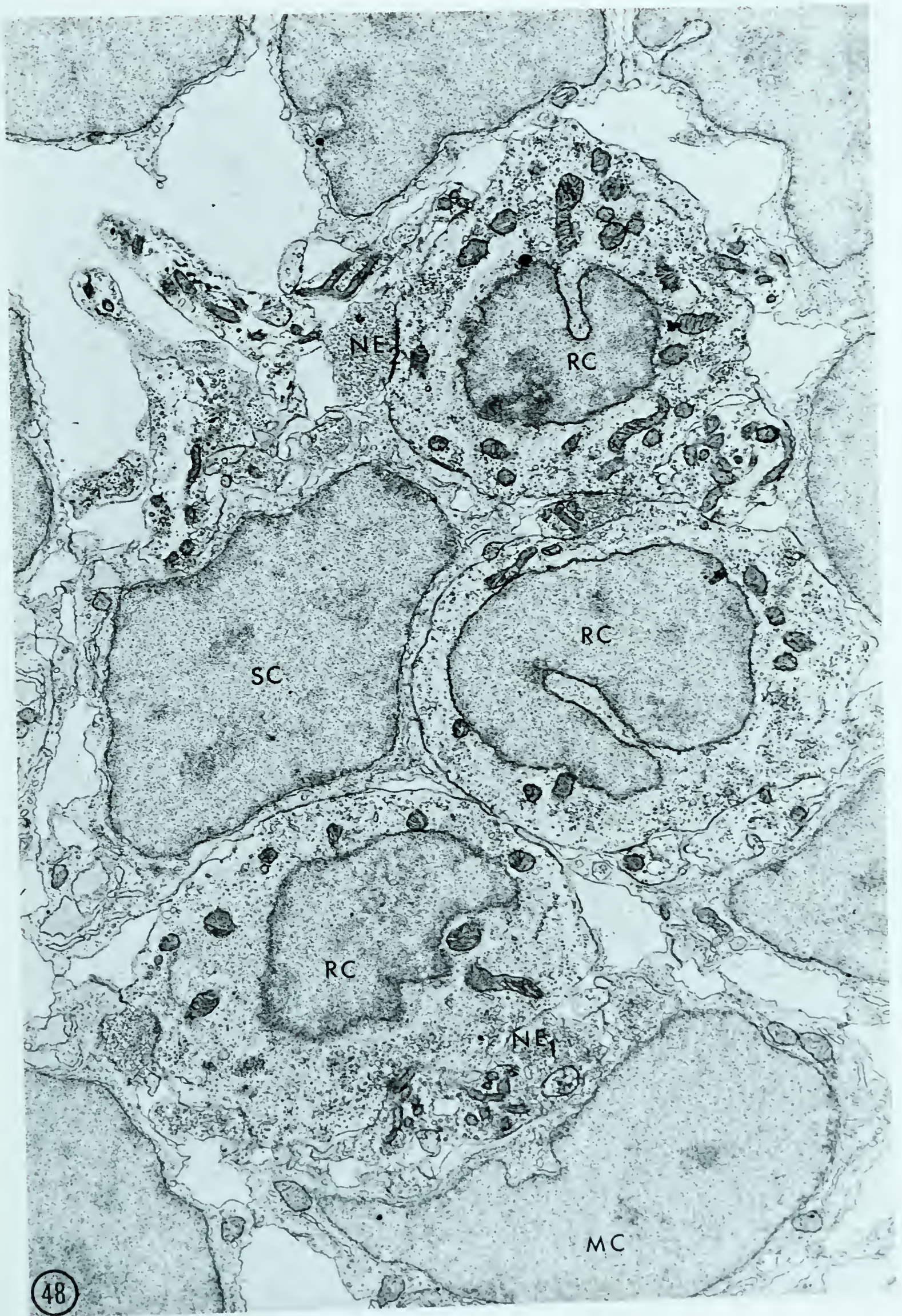


Fig. 49. A vertical section through a part of the lateral-line organ showing the organelles of sustentacular cell (SC) and mantle cell (MC) .

EC: epidermal cell; ECE: enlarged cisternae of endoplasmic reticulum; ER: endoplasmic reticulum; G: Golgi complex; IS: intercellular space; RC: receptor cell.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 21,000.

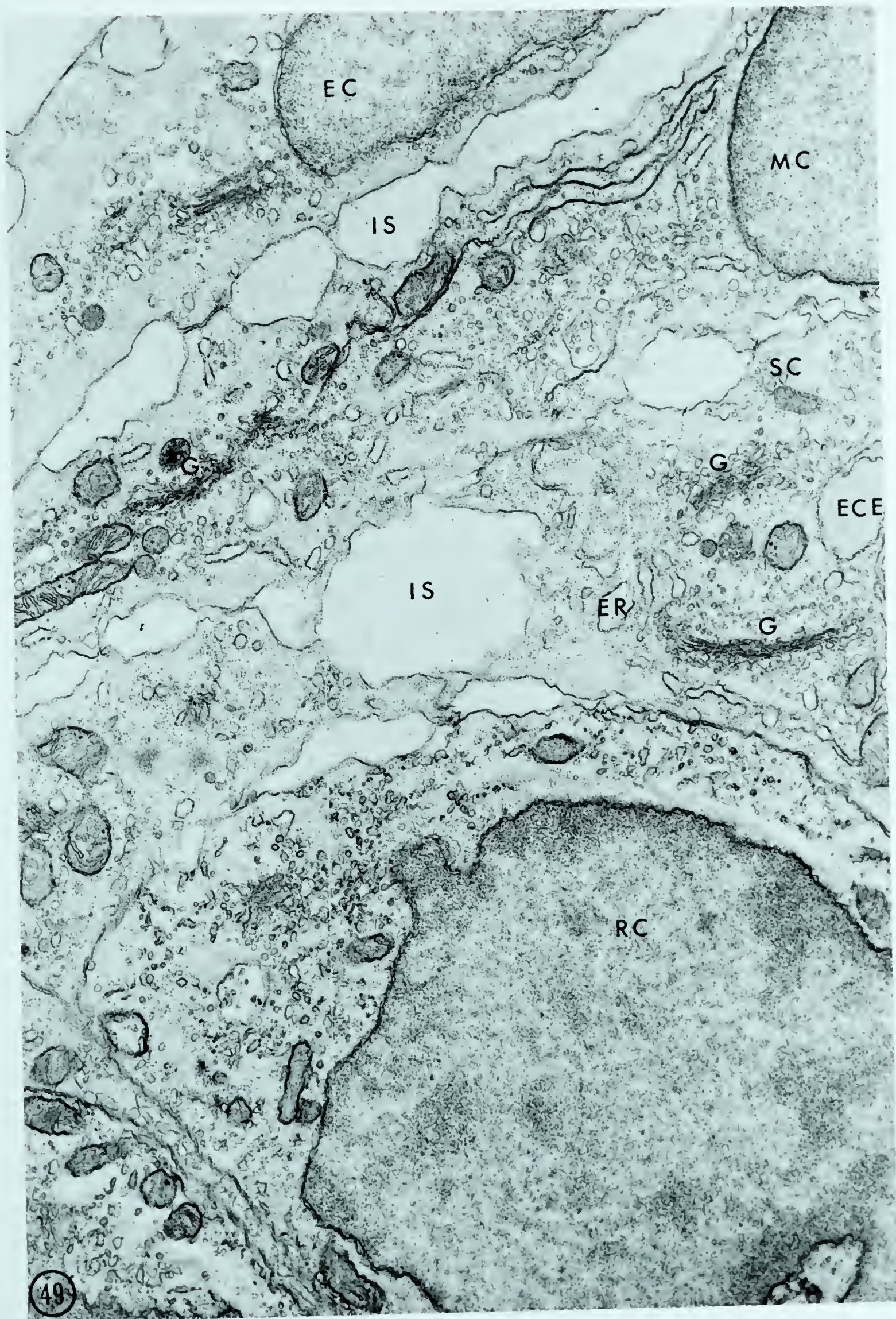


Fig. 50. A horizontal section through the base of the lateral-line organ showing basal cells (BC), nerve fibers (NF), endoplasmic reticulum, and secretory product (SP) in the intercellular space (IS).
Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 19,500.

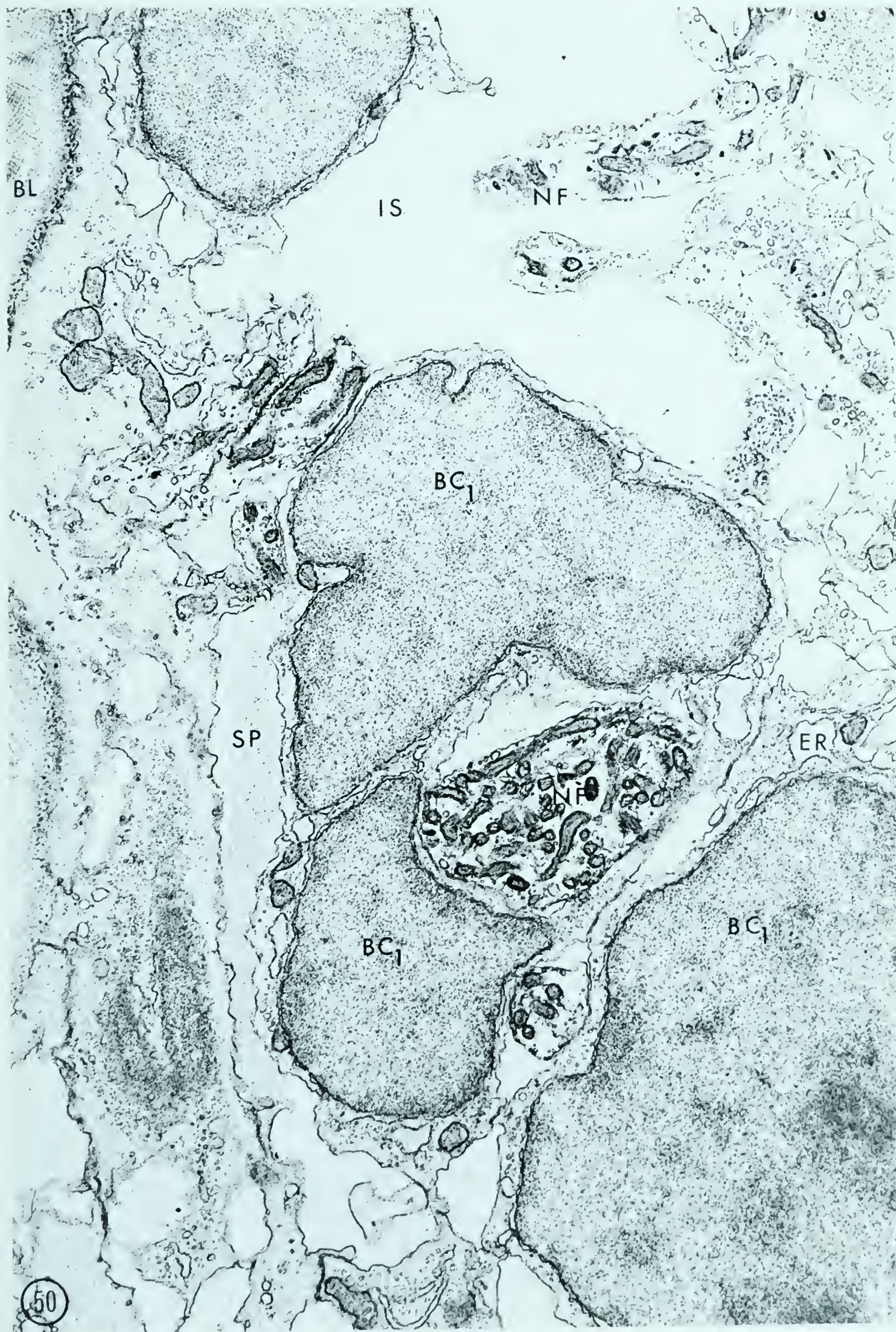


Fig. 51. A horizontal section showing the mantle cells (MC) which form a covering around the receptor cells (RC) and sustentacular cells (SC). There are also seen two epidermal cells (EC) around the organ. Osmium tetroxide fixation, Araldite embedding, and uranyl acetate staining. X 11,400.

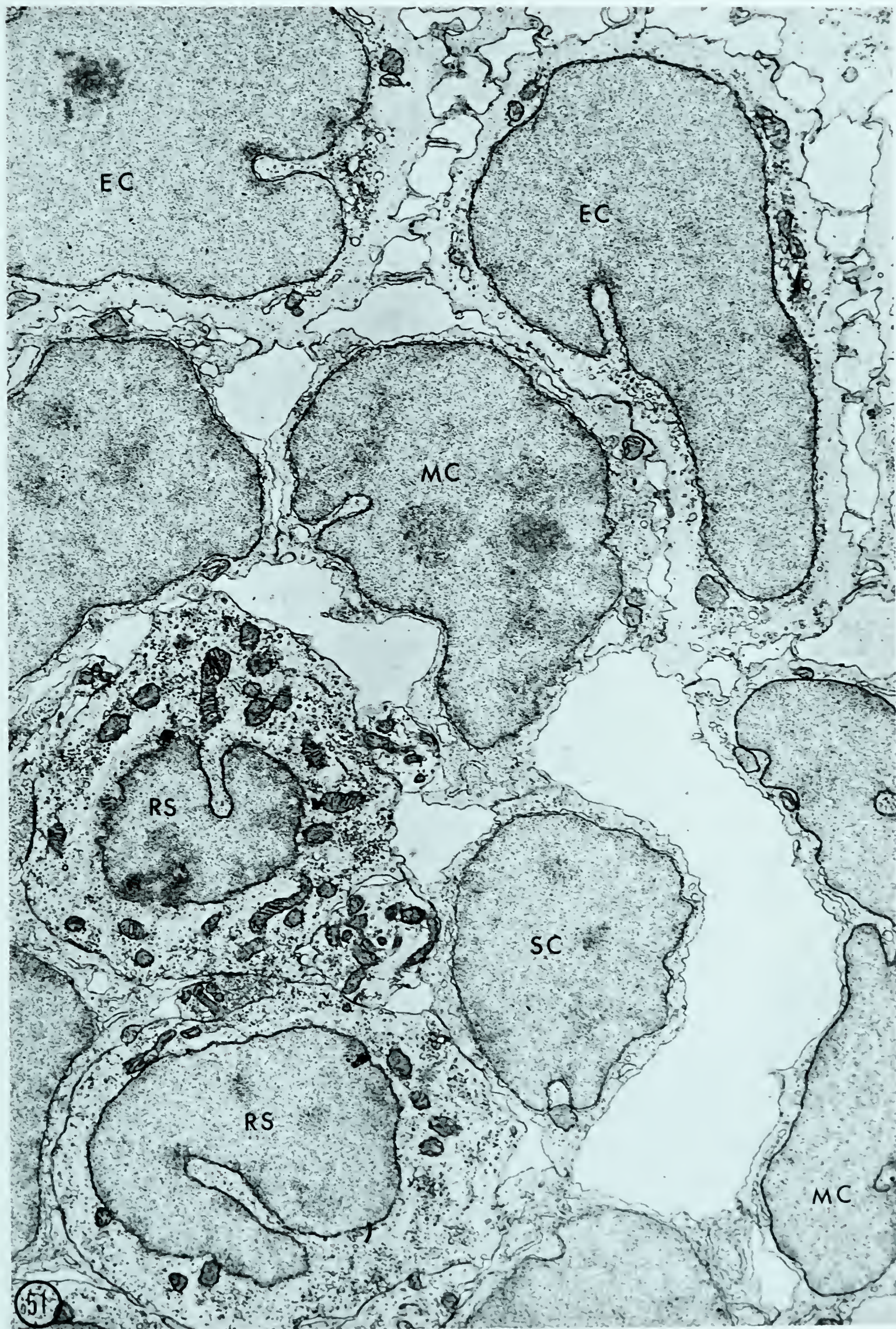


Fig. 52. A cross section of the lateralis nerve.
Both types of nerve fibers, myelinated
(MNF) and unmyelinated (UMF) are present.
Osmium tetroxide fixation, Araldite
embedding and lead hydroxide staining.
X 17,500.

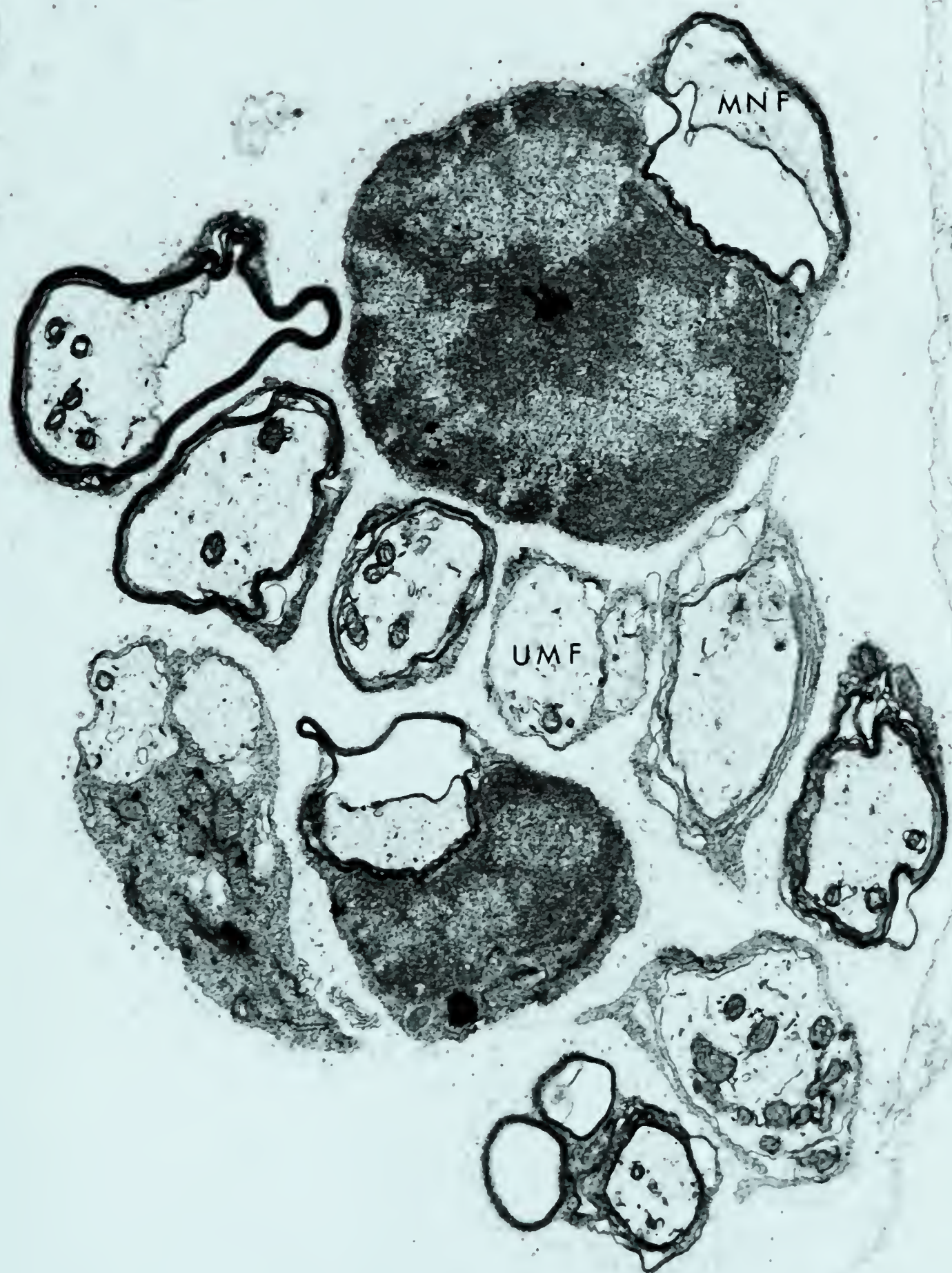


Fig. 53. A section through the basal region of the lateral-line organ showing nerve fiber (NF) surrounded by a Schwann cell (SchC) passing through the basement lamella (BL). The nerve fibers (NF) inside the organ are naked. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 21,000.

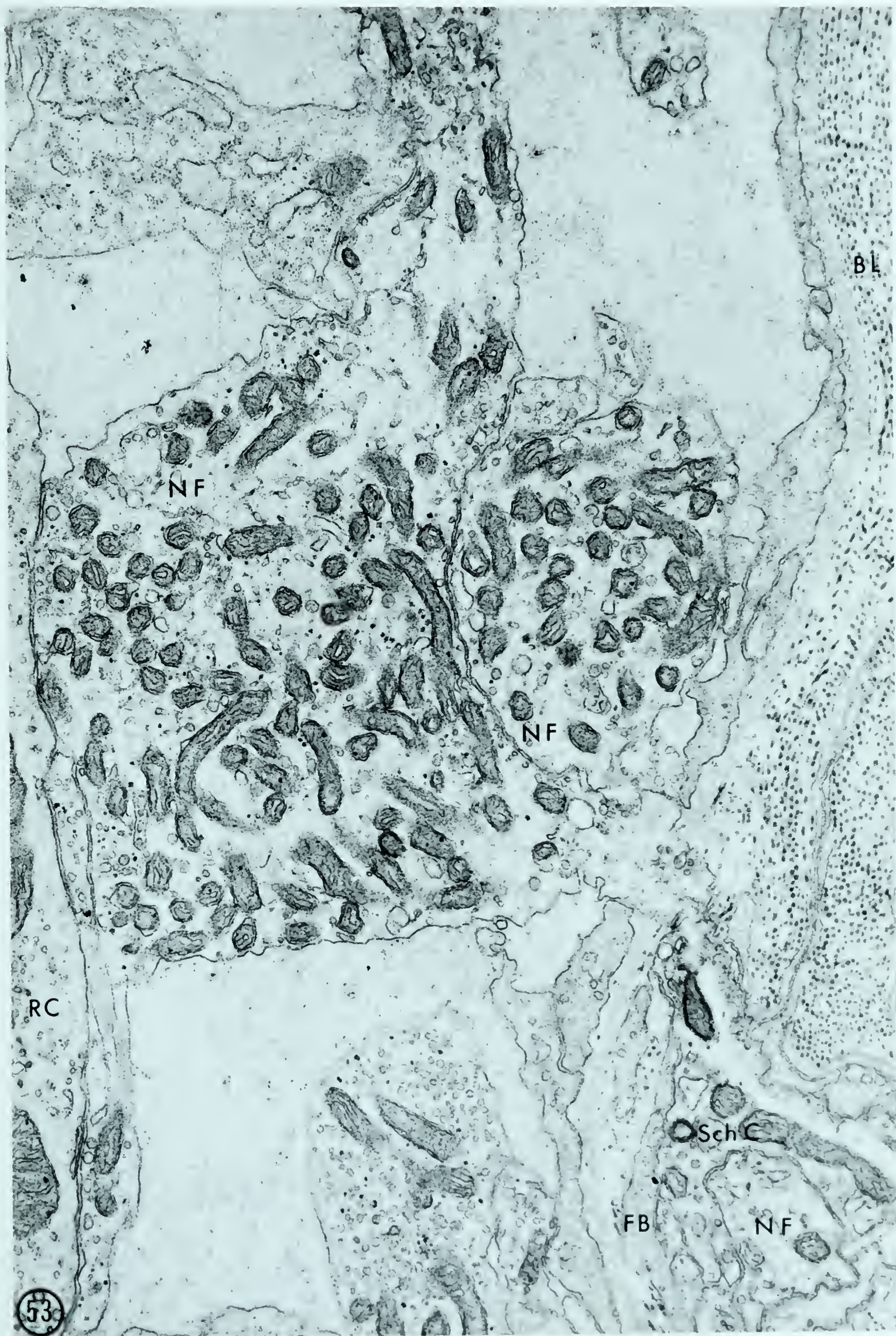


Fig. 54. A vertical section through the skin showing a cell (NC) in the act of migrating from the dermis into epidermis through the basement lamella (BL). It is also accompanied by nerve fibers (NF).

BC: basal cell; OC: outer cell of the epidermis.

Osmium tetroxide fixation, Araldite embedding and PTA staining. X 15,400.

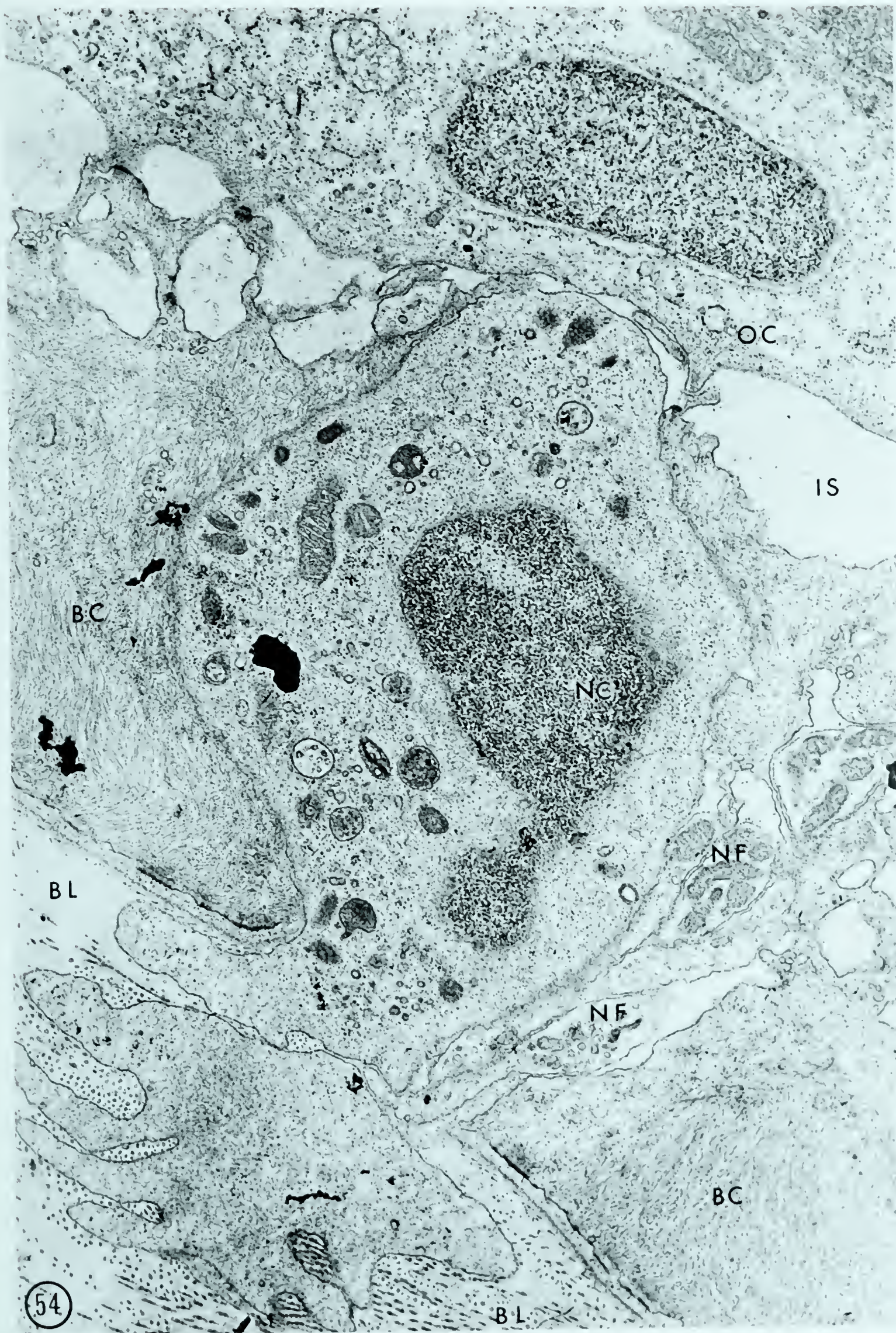


Fig. 55. A light microscope picture of epidermal melanophores. The thickest region in the center contains the nucleus. X 700.

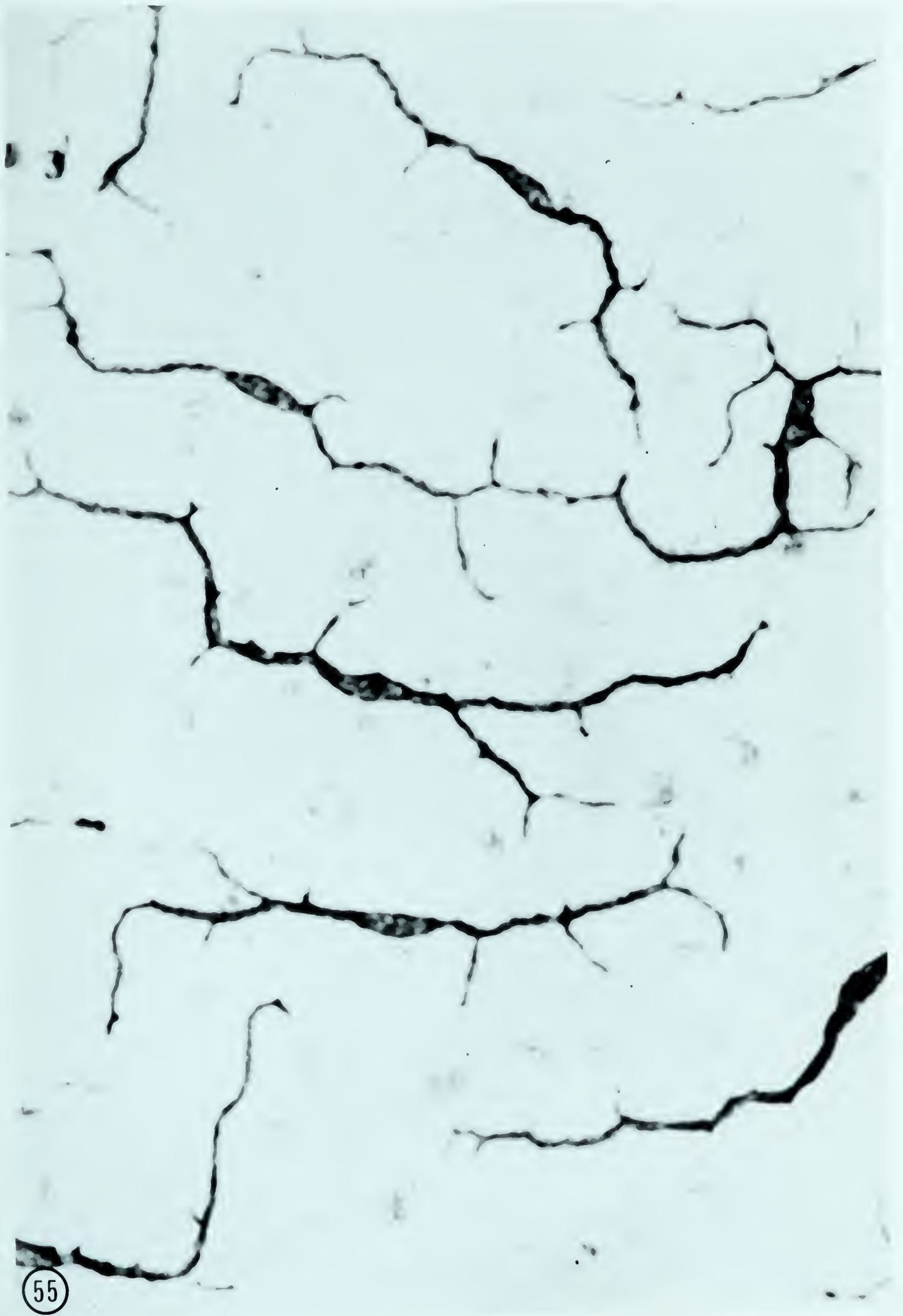


Fig. 56. A horizontal section through the basal epidermal cells. A portion of melanophore (MP) is seen lying in between the epidermal cells (EC).
Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining.
X 12,400.

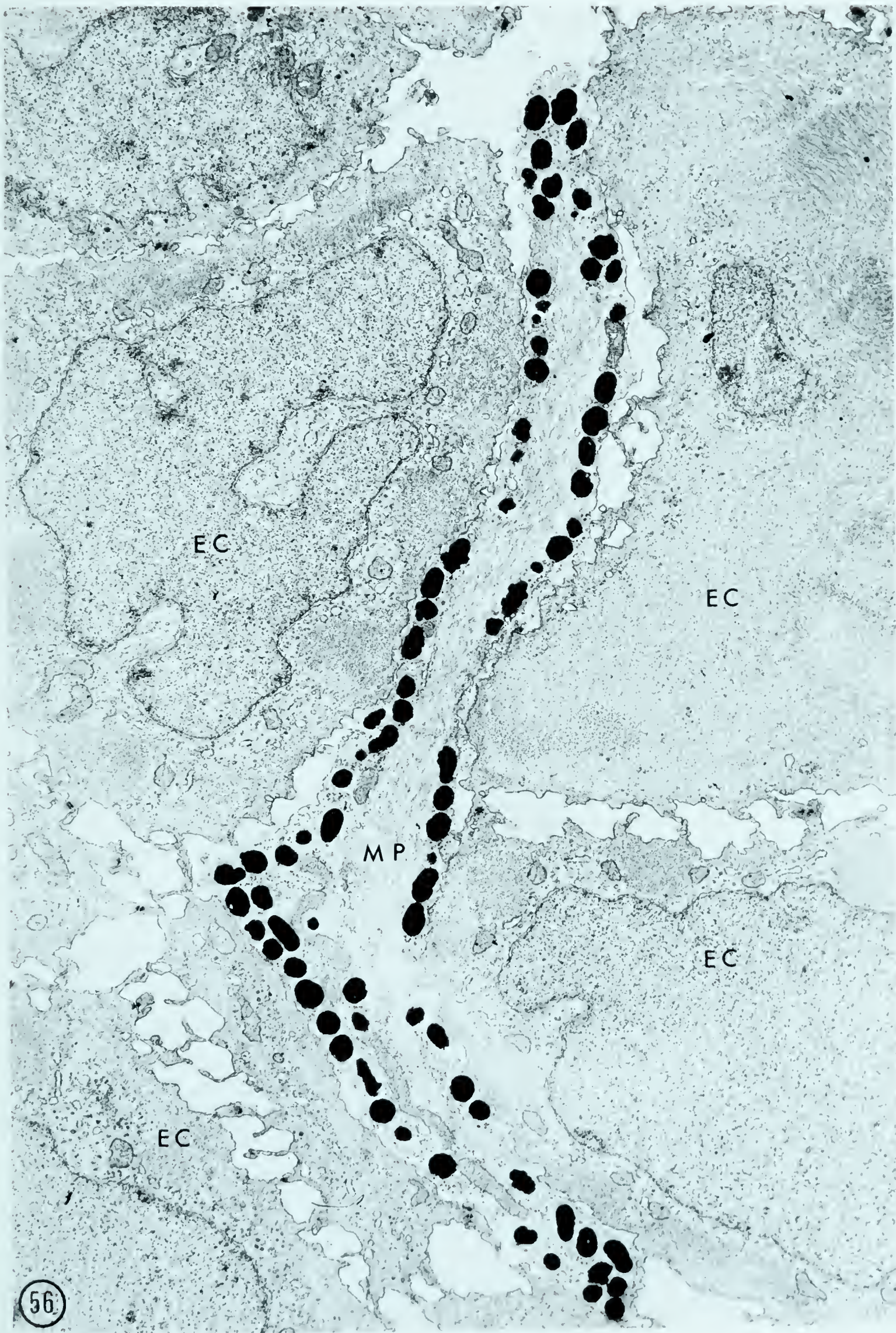


Fig. 57. An enlarged portion of figure 56 to show the keratin filaments (KF), ergastoplasm (ER), mitochondrion and intensely opaque melanin granules. The plasma membrane of the melanophore is not involved in any structural union with the surrounding epidermal cells. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 37,500.

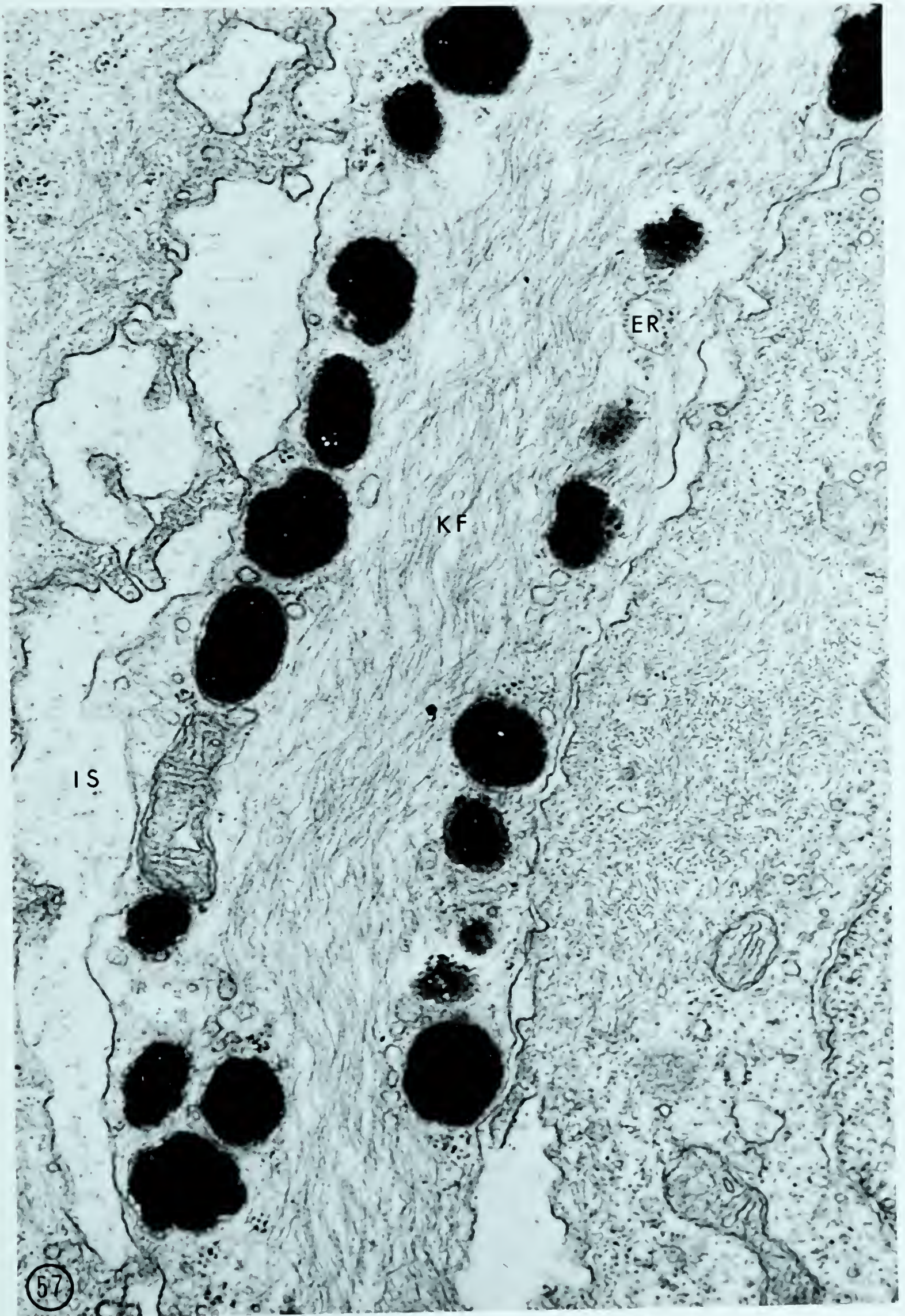


Fig. 58. A portion of epidermal melanophore, showing melanin granules, keratin filaments (KF) and cytoplasmic organelles, lying in the inter-cellular space of the epidermal cells (EC). Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 23,000.

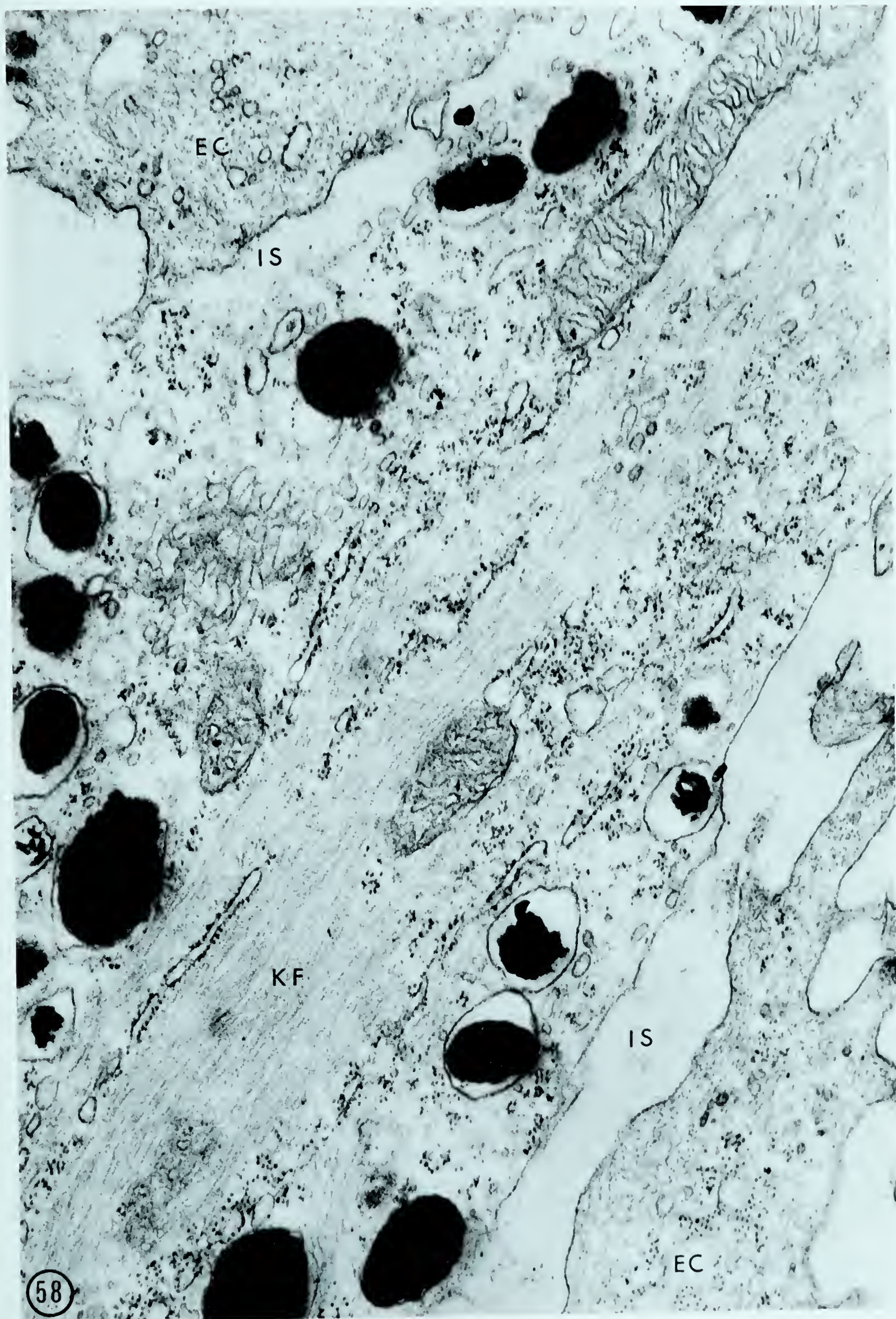


Fig. 59. A part of the epidermal melanophore showing melanin granules, keratin filaments (KF) and a microtubule (MT).
Glutaraldehyde-osmium tetroxide fixation,
Araldite embedding and uranyl acetate and
lead hydroxide staining. X 36,500.

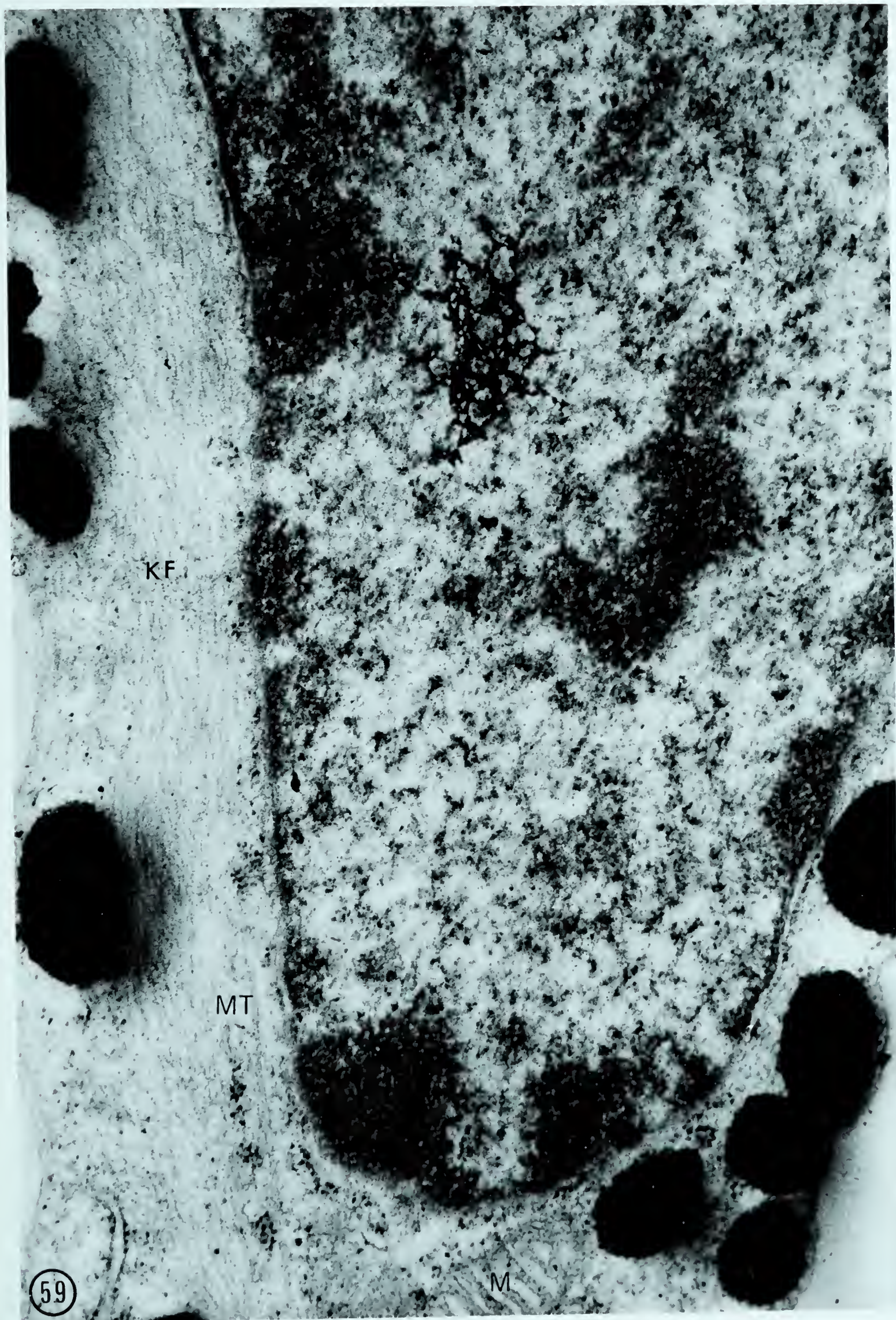


Fig. 60. A part of the melanophore at high magnification to show the keratin filaments (KF).

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining.
X 86,000.

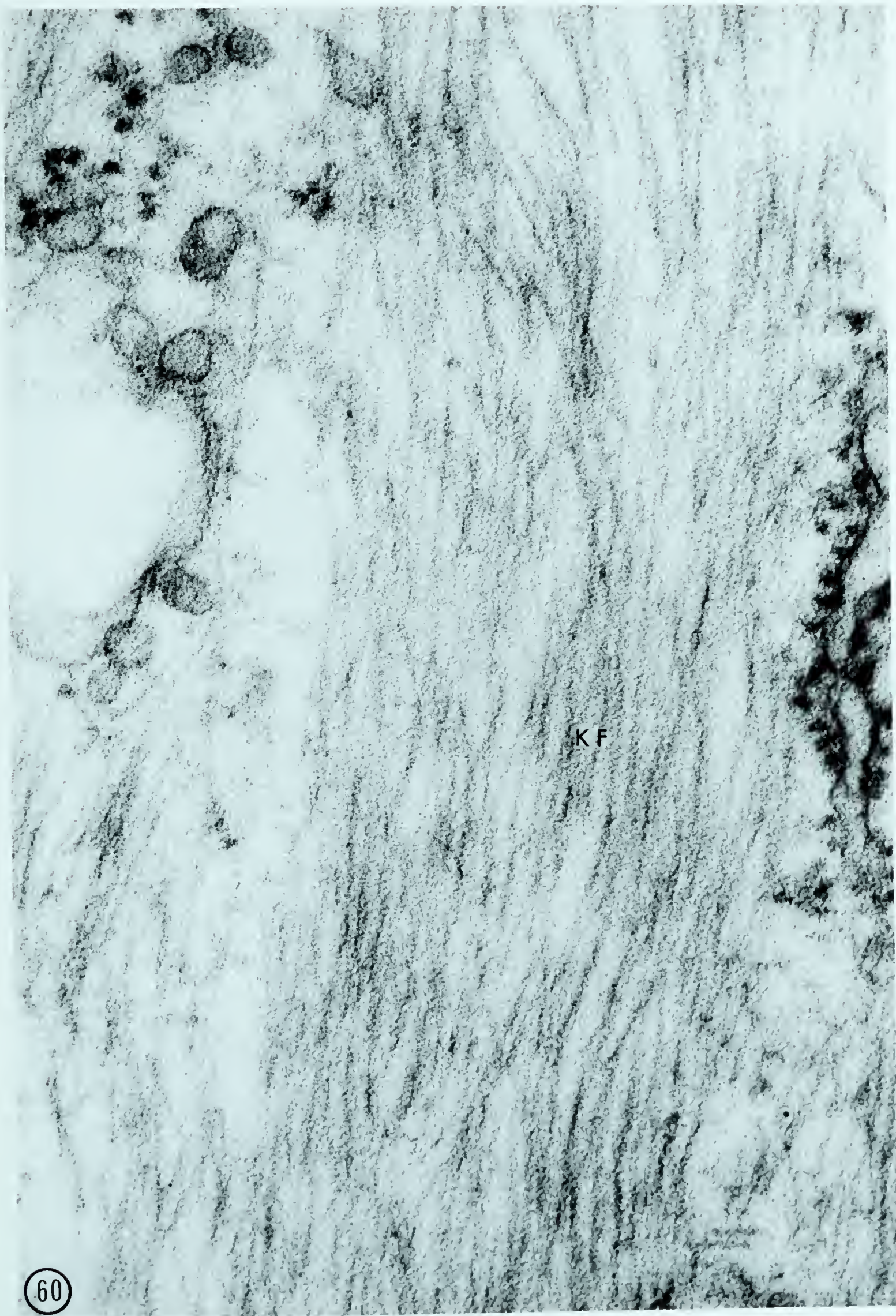


Fig. 61. A section through the nucleus of an epidermal melanophore showing various organelles such as mitochondria and Golgi complex (G) .
Osmium tetroxide fixation, Araldite embedding, uranyl acetate staining.
X 12,000.

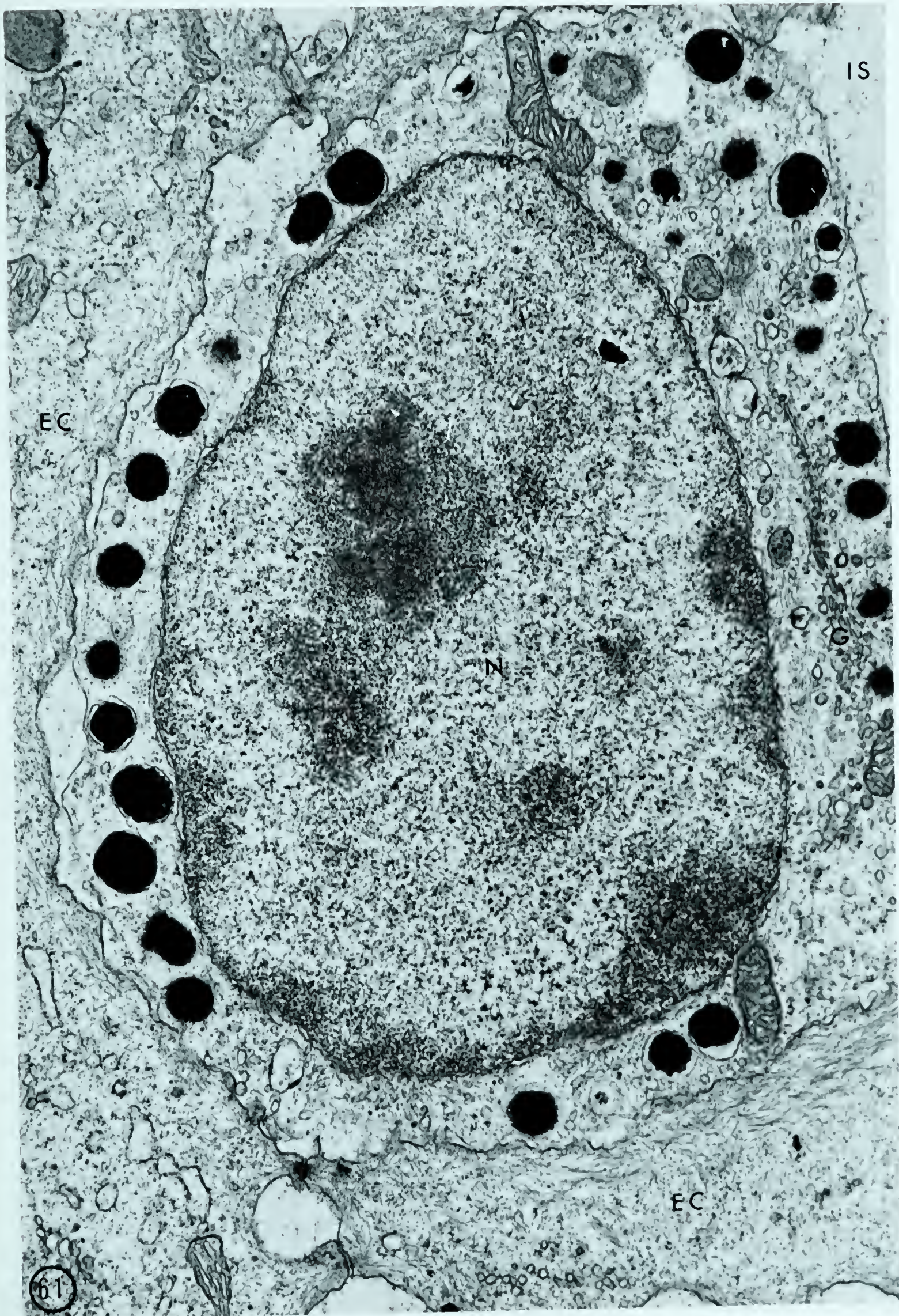


Fig. 62. A section through the middle of the epidermal melanophore showing lysosomes (LY) and dense bodies (DB) lying in the perinuclear region.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 30,800.



Fig. 63. Same as figure 62, showing the lysosomes
(LY) .

Osmium tetroxide fixation, Araldite
embedding and uranyl acetate staining.
X 35,000.



Fig. 64. Part of an epidermal melanophore lying in the intercellular spaces between the epidermal cells (EC) and showing dense bodies (DB) and lysosomes (LY). Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 13,200.

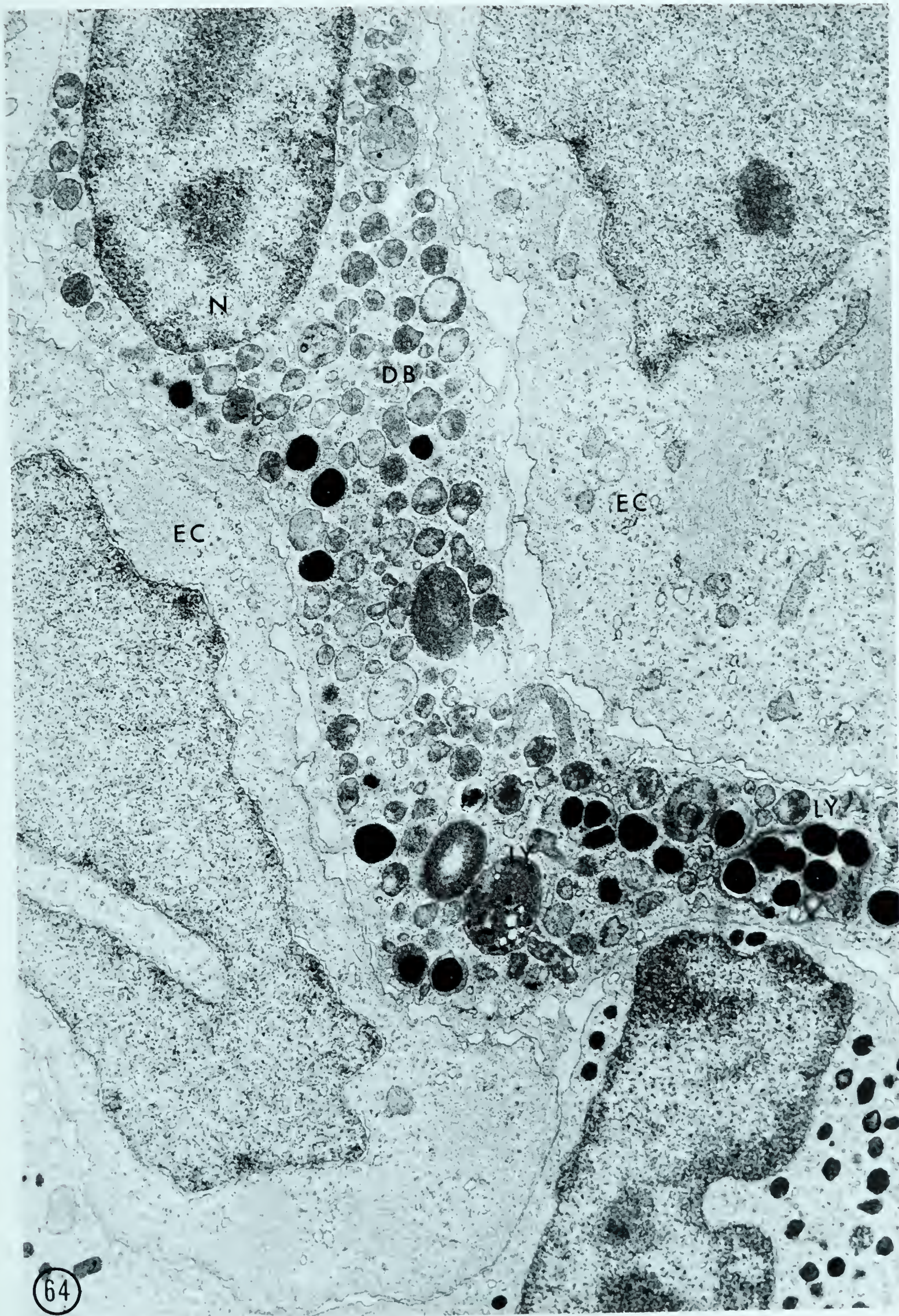


Fig. 65. Part of figure 64 enlarged to show the details in lysosomes (LY) and dense bodies (DB). Note the concentric lamellae in the large dense bodies.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining.

X 37,500.

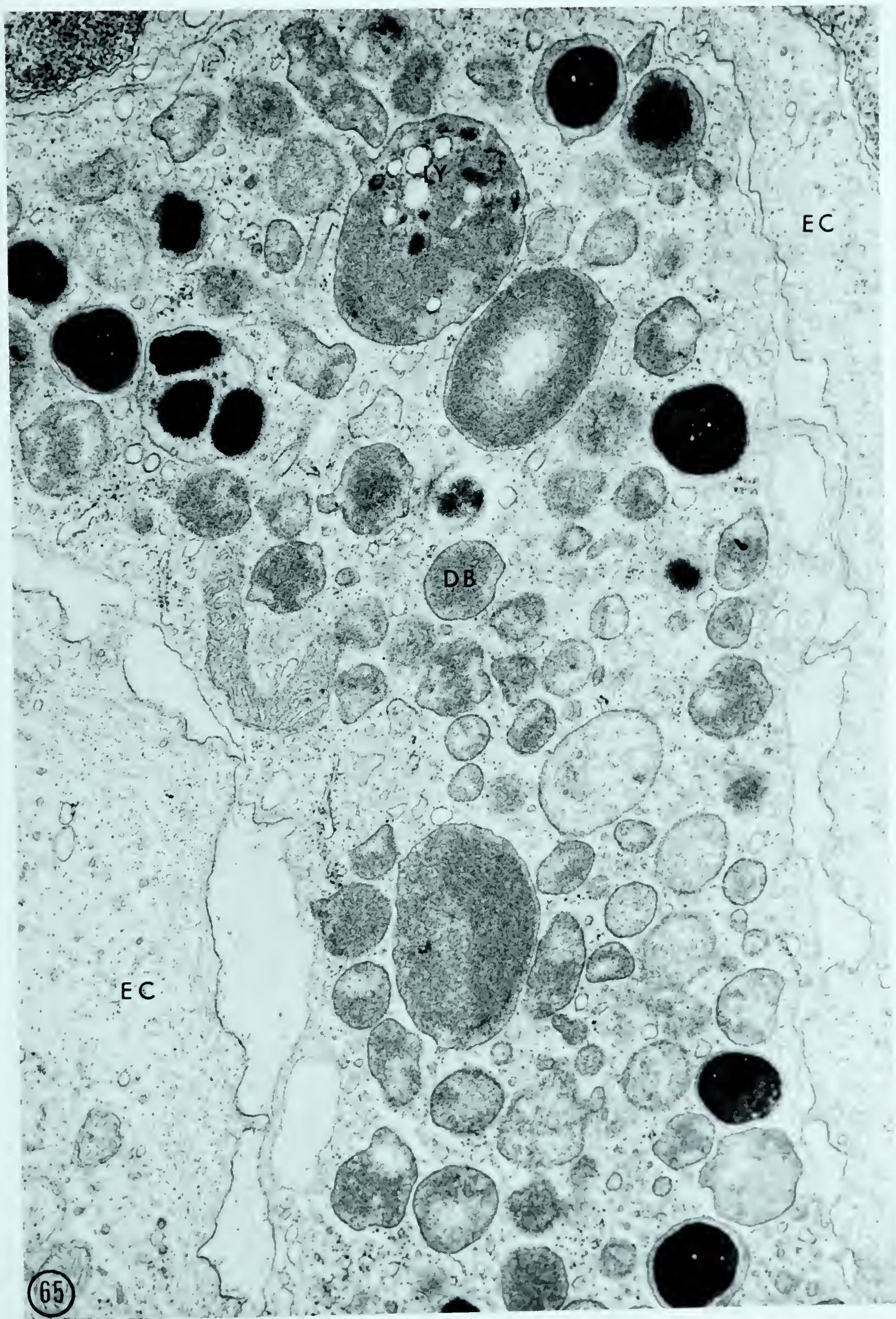


Fig. 66. A cross section through branch of the epidermal melanophore showing layered membranes in the lysosomes (LY). Osmium tetroxide fixation, Araldite embedding, and PTA staining. X 25,200.

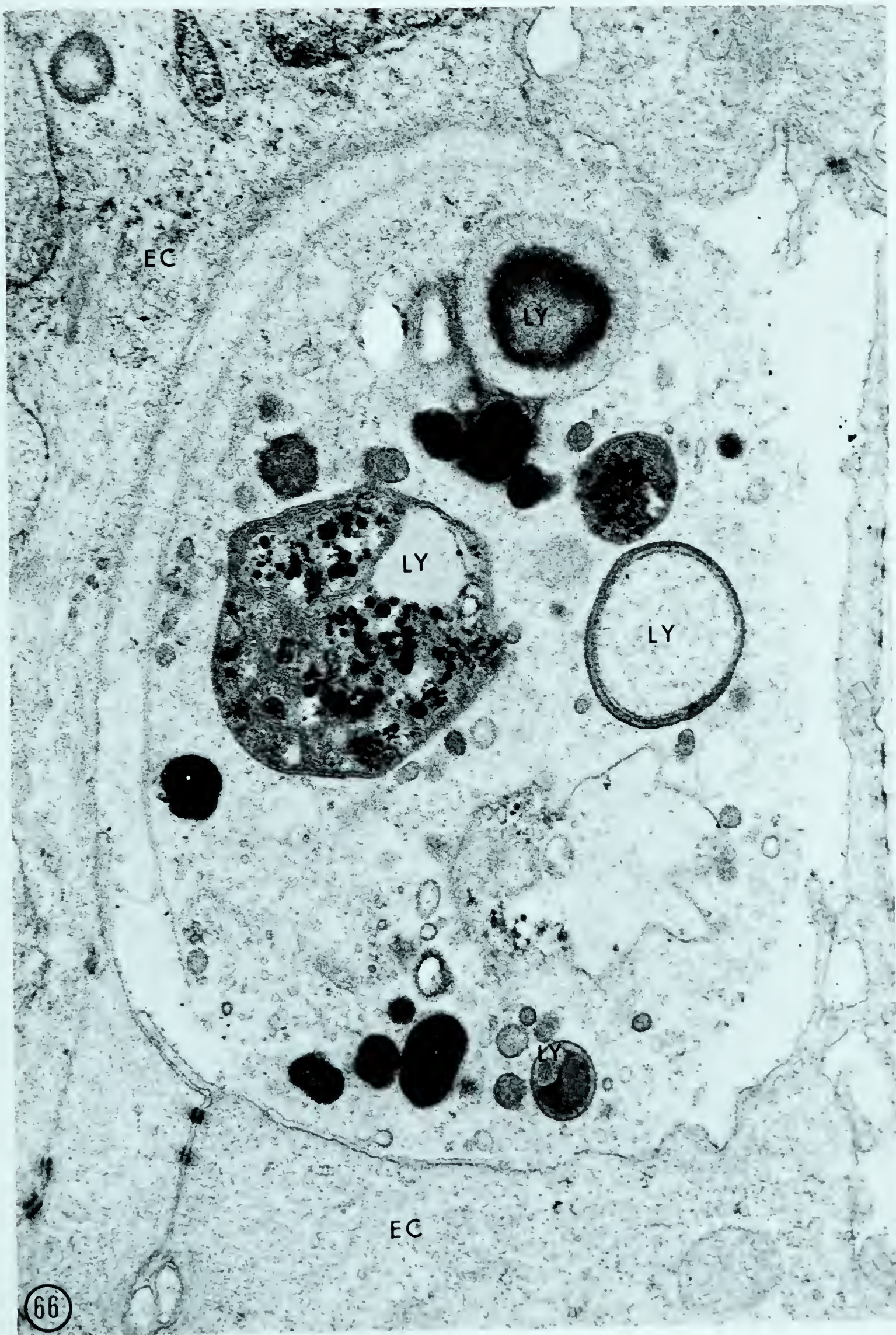


Fig. 67. A part of an epidermal melanophore showing a large dense area of cytoplasm which has melanin granules and membranes within it. It is perhaps a large lysosome. Osmium tetroxide fixation, methacrylate embedding and uranyl acetate staining. X 22,700.

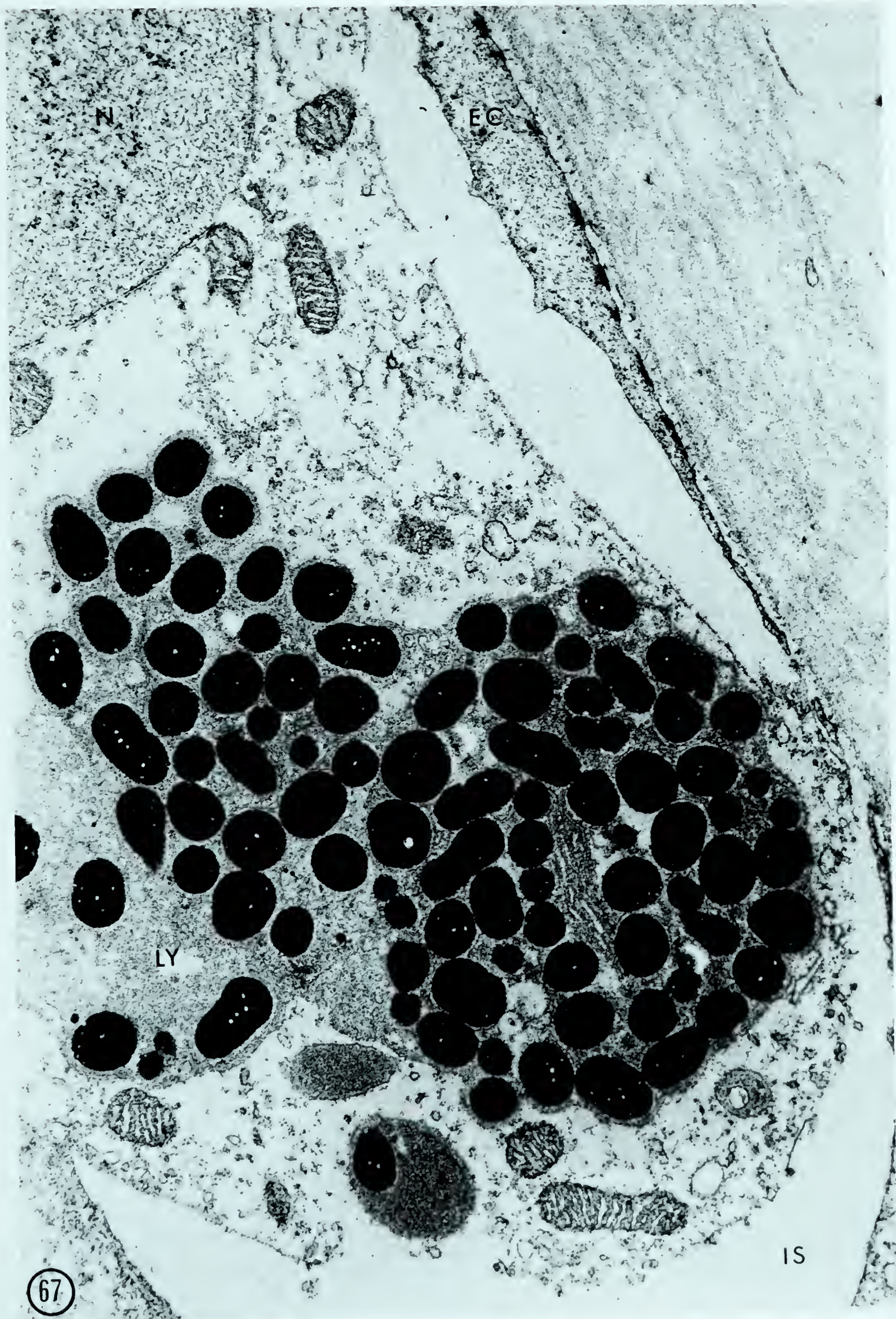


Fig. 68. A light micrograph of a dermal melanophore.
The light area in the center is the nucleus.
X 2,800.

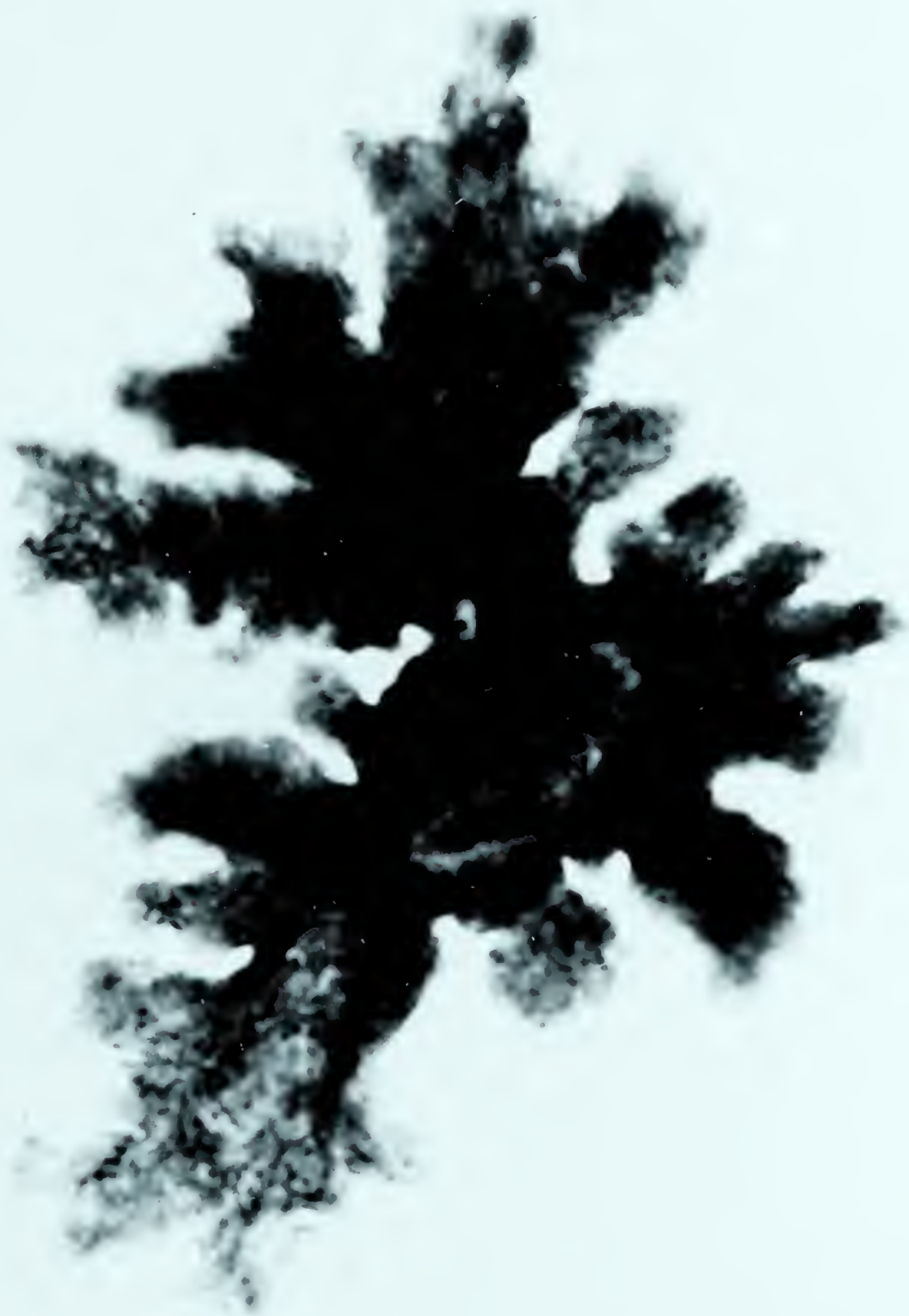


Fig. 69. A section through the dermal melanophore embedded in the dermal connective tissue. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 14,500.

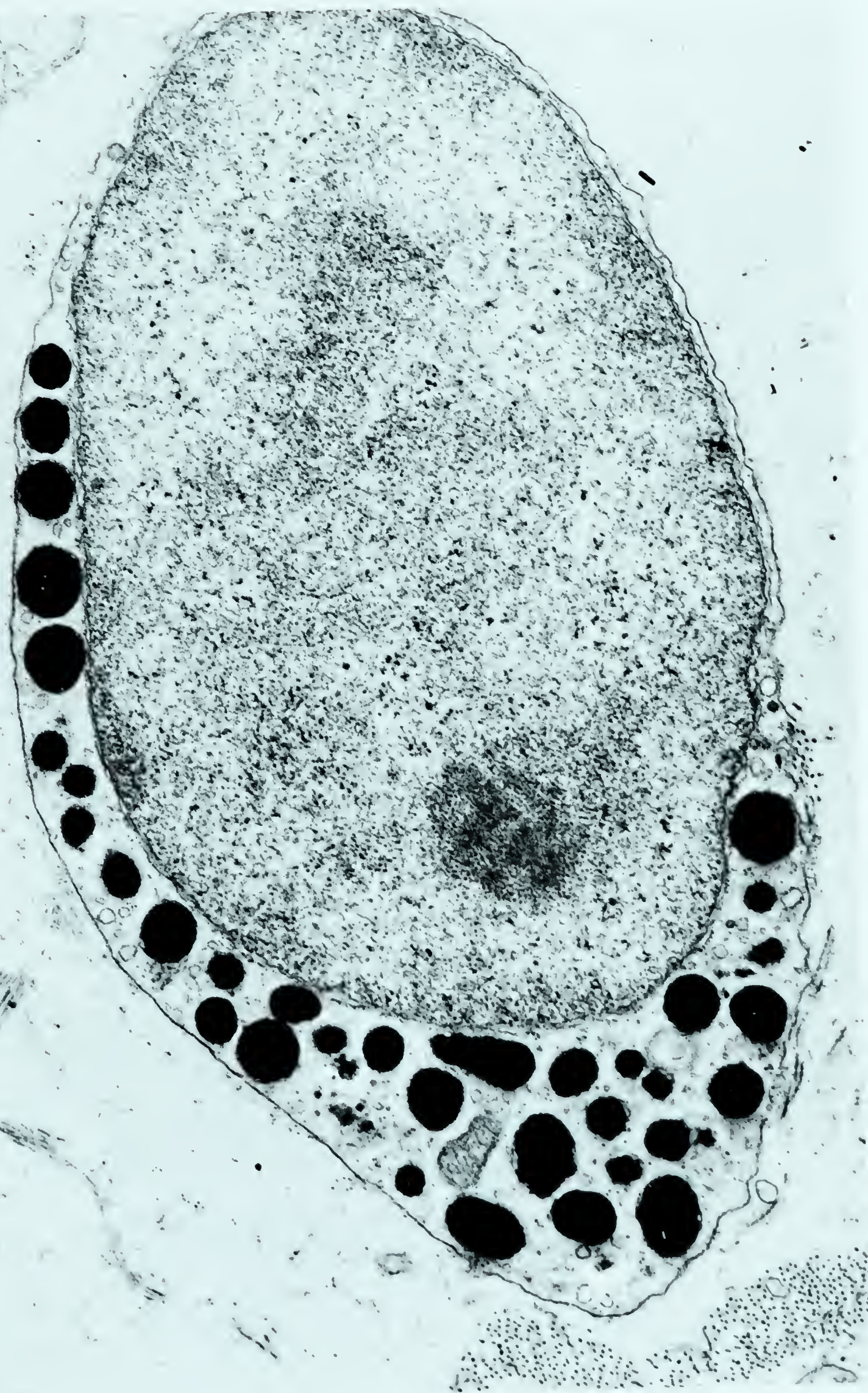


Fig. 70. Part of the dermal melanophore. Note each melanin granule being surrounded by a membrane and there is almost a complete lack of keratin filaments. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 27,000.





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